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REBECCA NIMRAH UMEED DE SOUZA

Impacts of the environment on the vocal behaviour of *Trichechus manatus manatus*

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Tese apresentada ao Programa de Pós-graduação em Biologia Animal da Universidade Federal de Pernambuco, como requisito parcial para a obtenção do título de Doutora em Biologia Animal. Área de concentração: Biologia Animal. Correspondente ao indicado na ata de defesa.

Orientadora: Profa. Dra. Bruna Martins Bezerra

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To Liliany, my light in the darkest of days, my inspiration in the most hopeless of days and my heart for the rest of my days.

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Finally, to my family for always encouraging me to dream big and to my dearest husband and daughter for believing in me when I was unable to believe in myself.

Never measure the height of a mountain until you reach the top, then you will see how low it was.

Dag Hammarskjold

RESUMO

Esta tese teve como objetivo investigar a complexidade da comunicação vocal dos peixes-boi marinhos, *Trichechus manatus manatus*, mantidos em centros de cativeiro e de reintrodução, e vida livre no Nordeste do Brasil. Além disso, a tese focou em detalhar como fatores abióticos influenciam essa complexidade vocal e propagação de vocalizações. A tese foi dividida em três partes principais. A primeira parte trouxe uma revisão bibliométrica para elucidar o estado da arte sobre os potenciais efeitos dos fatores abióticos na ecologia comportamental e na comunicação vocal de mamíferos marinhos. A segunda parte focou em elucidar a complexidade do repertório vocal dos peixes-boi marinhos e a comunicação entre indivíduos. Por fim, a terceira parte focou em elucidar os efeitos dos fatores abióticos na produção vocal e na propagação de vocalizações dos peixes-boi marinhos. Foi possível registar a produção de quatro tipos de vocalizações pelos peixes-boi estudados: Squeaks, Chirps, Trills e Pulse. A vocalização Pulse foi descrita pela primeira vez para essa espécie nesta tese. A estrutura física das vocalizações Squeak produzidas por peixes-boi apresentaram diferenças a nível de indivíduo, sugerindo a produção de vocalizações assinaturas para a espécie. Foi constatado que pares e trios de mães e filhotes se comunicam com vocalizações com estruturas físicas únicas. A taxa de vocalizações dos peixes-boi marinhos estudados foi idiossincrática de acordo com o período do dia e o nível da maré. No entanto, a estrutura das vocalizações não diferiu o suficiente para diferenciá-las com base no período do dia ou o nível da maré. O comportamento da estrutura das vocalizações de peixes-boi variou em termos de propagação de acordo com o sexo e a origem do animal vocalizador (cativeiro ou reintrodução). Fatores abióticos como pH, salinidade e temperatura influenciaram a propagação das vocalizações. Por exemplo, houve correlação positiva entre o pH e a amplitude relativa para vocalizações de fêmeas em cativeiro durante a propagação. Também foi encontrada correlação positiva entre temperatura e largura de banda para vocalizações de machos e filhotes em cativeiro. Uma correlação negativa ocorreu entre o pH e a duração para vocalizações de machos mais velhos. Em geral, os resultados desta tese reforçam que as vocalizações dos peixes-boi desempenham um papel importante na comunicação vocal dos mesmos e provavelmente contêm informações sobre a identidade individual, facilitando o reconhecimento entre indivíduos da mesma espécie. A tese também mostra que os peixes-boi alteram o padrão de suas chamadas de acordo com a hora do dia e/ou nível da maré, mas conservam a estrutura física das vocalizações nessas situações. As vocalizações dos peixes-boi sofrem pouca degradação com a distância em piscinas naturais em áreas recifais sob diferentes condições de pH, temperatura e salinidade – um potencial reflexo da adaptação desses animais em áreas estuarinas que que sofrem com alterações constantes dessas variáveis. Assim, as mudanças previstas dessas variáveis causadas pelas mudanças climáticas provavelmente pouco afetarão a propagação de vocalizações, e consequentemente, a comunicação vocal do peixe-boi marinhos. No entanto, é possível que o aumento previsto na frequência de tempestades e inundações possa impactar mais fortemente essa subespécie.

Palavras-chave: Estrutura de vocalização; Produção de vocalização; Propagação de vocalização; Sirenia; Flexibilidade de vocalização.

ABSTRACT

This thesis aims to investigate the complexity of Antillean manatee, *Trichechus manatus manatus,* vocal communication in North-eastern Brazil. Additionally, this these aimed to detail how abiotic factors influence the vocal complexity and vocalisation propagation of this sub-species. The first part of this thesis is comprised of a bibliometric literature review, with the aim of exploring the available published literature on the effects of abiotic factors on the behavioural ecology and vocal communication of marine mammals. The second part focused on elucidating the complexity of the vocal repertoire of Antillean manatees and conspecific communication. Finally, the third part of this thesis focused on demonstrating the effects of abiotic factors on the vocal production and propagation of Antillean manatees. It was possible to register four call types in this thesis: Squeaks, Chirps, Trills and Pulse calls. The pulse call type was described for the first time here. The physical structure of the Squeak vocalisations presented differences at the individual level, suggesting the production of signature vocalisations by this sub-species. Communication between mother and calf manatees deserves particular attention, since we found that free-living mother-calf pairs and trios communicate using vocalisations with unique physical structures. Call rates of Antillean manatees were idiosyncratic according to time of day and tide level. However, call structure did not differ enough to differentiate the calls based on these factors. The behaviour of manatee call structures varied in terms of propagation, based on sex and animal origin (captive or reintroduction centre). Abiotic factors such as pH, salinity and temperature influenced call propagation. For example, there was a positive correlation between pH and relative amplitude for captive female calls during call propagation. There was also a positive correlation between temperature and bandwidth for captive male and calf calls. A negative correlation was found between pH and duration for older male calls. Overall, the results of this thesis reinforce that Antillean manatee vocalisations play an important role in vocal communication and likely contain information on individual identity, facilitating conspecific recognition. This thesis also showed that manatees alter their call pattern based on the time of day and/or tide level, but conserve vocalisation structure under these conditions. Manatee vocalisations suffer from little degradation over distance in natural pools in reef areas under different pH, temperature and salinity conditions. This may reflect an adaptation of these to animals to estuarine areas which experience constant abiotic alterations. Thus, the predicted changes in pH, temperature and salinity, caused by climate change will likely have little effect on Antillean manatee call propagation and consequently, their vocal communication. However, it is possible that the predicted increase in storm and flood frequencies may impact this sub-species more strongly.

Keywords: Call structure; Call production; Call propagation; Sirenia; Call flexibility.

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Article 3 - Tidal and diel influence on the Antillean manatee, *Trichechus manatus manatus*, vocalisations in North-eastern Brazil

Article 4 - effects of abiotic factors on Antillean manatees (*Trichechus manatus manatus*) call propagation through playback experiments.

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1 INTRODUCTION

Global sea surface temperatures have increased by 0.014 (±0.001)°C per year (von Schuckmann et al., 2018; Affatati et al., 2022), culminating in an increase of 0.88°C since the beginning of the 20th century. This is predicted to increase by 0.86-2.89°C by 2100 (IPCC, 2022). Furthermore, since the 1980s, surface ocean pH has declined by a rate of 0.016-0.020 per decade, decreasing from 8.1 to 8.2 since the pre-industrial era (IPCC, 2022). Projected pH decreases are estimated as between 0.17±0.003 to 0.37±0.007 by 2100 (IPCC, 2022).

Sound attenuates naturally in the ocean. However, this natural attenuation can be affected by changes in ocean pH (Kloepper and Simmons 2014). Ocean pH determines the concentration of chemicals that absorb sound near frequencies of 1kHz (Brewer 1997; Hester et al., 2008; Ilyina et al., 2009). Sound absorption decreases with decreasing pH, and as such, predictions have estimated a possible decrease of 60% in the sound absorption of frequencies between ~100Hz and 10kHz in high latitudes by the year 2100 (Ilyina et al., 2009). Sound absorption also decreases with increasing temperatures, and with the predicted increase in sea surface temperature, the sound absorption coefficient may decrease by up to 8% for frequencies below ~10kHz (Hester et al., 2008; Ilyina et al., 2009).

A decrease in sound absorption in the oceans will result in both natural and anthropogenic sounds travelling further, thereby increasing noise levels in the environment (Kloepper and Simmons 2014; Gazioglu et al., 2015). These alterations in the marine environment may negatively affect marine mammal acoustic behaviours. However, these predictions have not been empirically tested in the ocean and are based on theoretical calculations (Kloepper and Simmons 2014). Indeed, to date, there has been one published quantitative study on the expected magnitude and distribution of climate-change-induced sound speed variations, and the impacts of changes in sound propagation on crucial marine mammal activities have not yet been assessed (Affafati et al., 2022).

The soundscape of the marine environment is constantly changing and adapting to changes caused by anthropogenic impacts, such as climate change (Gazioglu et al., 2105; Miksis-Olds et al., 2018). A soundscape can be defined as the sum of multiple sound sources arriving at the location of a receiving animal or recorder in an environment and is an important subject of “acoustic ecology” (Miksis-Olds et al., 2018). Coastal ocean soundscapes may be affected by climate change through changes in surface sound scattering loss caused by potential changes in wind patterns, changes in fauna and shipping patterns, and the movement and shape changes of coastal temperature fronts (Lynch et al., 2018).

The speed of sound in the ocean generally ranges from 1450m/s- 1550m/s (Seghal et al., 2010). However, in reality, it depends upon several factors such as temperature, salinity and pH (Seghal et al., 2010, Lynch et al., 2018). In other words, the speed of sound increases as temperature, pressure and salinity increase (Mercado and Frazer, 1999). Underwater acoustic signals, when transmitted, suffer a reduction in acoustic intensity over distance due to several variables affecting propagation factors in the ocean (Seghal et al., 2010). This reduction in acoustic intensity is known as propagation loss and is a consequence of three main factors: attenuation, refraction and geometrical spreading (Seghal et al., 2010).

Marine mammals utilise sound in almost all aspects of their life, such as during feeding, reproduction, territory defence, and to warn others of danger (Au and Hastings, 2008; Hildebrand, 2009; Affatati et al., 2022). Such sounds are essential in facilitating information exchange, interactions and behaviour coordination (La Manna et al., 2019). Cetaceans, for example, have evolved to become acoustically oriented and developed highly specialised auditory systems, and consequently require adequate acoustic environments to survive (Wartzok et al., 2022; Affatati et al., 2022). However, an individual’s ability to use sound is strongly constrained by environmental factors (Mercado and Frazer, 1999). Where sounds travel, and the extent to which they will be distorted depend on the medium through which they are propagated, which can often be reflected in species’ repertoires (Mercado and Frazer, 1999).

Antillean manatees, *Trichechus manatus manatus,* are currently threatened by extinction and occur along the coast of North-eastern Brazil (MMA, 2014). Individuals produce vocalisations with fundamental frequencies ranging from 0.64kHz-8.1kHz and contain information on individual identity (Sousa-Lima et al., 2008; Chavarría et al., 2015; Umeed et al., 2018; Umeed et al., 2023). Relatively little is known about manatee vocal patterns and usage, which is concerning since these animals face a context of threat of extinction. Furthermore, as Antillean manatees are found in estuarine and coastal areas, changes in the abiotic factors could strongly impact individual survival.

Thus, here in this thesis, I aimed to: i) investigate if there is complexity in the use and structure of the vocalisations produced by *Trichechus manatus manatus,* identifying the potential information contained in the vocalisations (e.g., information about individual identity and the sex and age of the vocalising individual); ii) Investigate if there are patterns in the production of manatee vocalisations in captivity and reintroduction centres according to factors such as illumination period (day vs. night) and variation in tide level and iii) Investigate the effects of abiotic variables (i.e., salinity, acidification, and water temperature) on the propagation of different types of manatee vocalisations. To achieve this, I divided this thesis into two parts: Part I focuses on describing the vocal repertoire of Antillean manatees and vocal interactions between mothers and calves; and Part II describes the effects of abiotic factors on the vocal patterns and call propagation of captive and reintroduction manatees. Overall, I aimed to identify whether vocal production differs based on housing condition, age and sex and to investigate whether individuals produce calls containing information on individual identity. Additionally, I aimed to provide insight into manatee call production regarding the time of day and tide cycle, information which has not been previously published to our knowledge. Finally, I sought to provide information on manatee call propagation over distance.

The results of this thesis provide valuable insight into Antillean manatee vocal behaviour. Here I suggest several practical applications of our results in order to facilitate manatee conservation and minimise the number of calf strandings in North-eastern Brazil.

2 Literature Review - Impacts of environmental variables on marine mammal behaviour and vocal ecology: A systematic and bibliometric review.

2.1 Introduction

Sea-surface temperatures have increased by 1°C since pre-industrial times (Kennedy et al., 2019), with new estimates showing a high likelihood of ocean heat uptake of 9.2 ± 2.3 ZJ yr^{-1} to 12.1 ± 3.1 ZJ yr^{-1} from the years 1993-2017 (Johnson et al. 2018; Bindoff et al., 2019 Furthermore, atmospheric carbon dioxide concentration has increased by 31% since 1750 (IPCC 2001) resulting in a 25% increase in ocean acidity (i.e., a decrease in pH of 0.1 units) (IPCC 2014), with predictions estimating a total decrease of between 0.036–0.291 pH units by 2081–2100 (Bindoff et al., 2019). This decrease in pH corresponds to an increase in hydrogen ion concentration that is three times greater than that of pre-industrial times (Royal Society 2005).

The effects of climate change on marine mammal behavioural ecology have been well-documented from the beginning of the century, however, there is a distinct lack of understanding on the potential impacts of anthropogenically-induced climate change on marine mammal vocal behaviour. This is alarming as vocal communication plays a highly important role in the survival of many marine mammal species.

Vocal communication involves the emission and reception of acoustic signals, conveying information such as danger, resources, kinship and individual identity (Halliday and Slater, 1983; Bradybury & Vehrencamp, 1998). The effective range of vocal communication in marine environments depends on the characteristics of the environment that affect propagation loss, vocalisation frequency and amplitude and receiver hearing sensitivity (Miksis-Olds et al., 2009). Thus, callers must successfully calculate the frequency, amplitude and duration parameters of their calls in order to effectively transmit information. This is especially complex in the marine environment due to constantly fluctuating environmental conditions and the impact they have on underwater sound propagation (Seghal et al. 2010; Peng et al. 2015). This suggests that individuals may be required to alter their vocal production in accordance with their surrounding environmental conditions, in order to successfully communicate with conspecifics (Umeed et al., 2023).

Here we aimed to gather information on the documented and predicted impacts of environmental variables, with focus on climate change variables and anthropogenic noise, on marine mammal behavioural ecology and sound propagation. To achieve this, we performed a systematic and bibliometric literature review in order to:

i) Understand trends in research publication, by considering focal species, country of publication and citation impact and

ii) Identify potential gaps in knowledge on the impacts of changing environmental factors on marine mammal communication and how this may impact species' conservation.

2.2 Data and methods

We performed a literature review using the platforms "Google Scholar" and "PubMed" to gather published research using the terms, "climate change and marine mammals", "sound propagation and climate change", "climate change affects acoustic ecology", "effect of abiotic environmental factors on cetacean communication" and "influence of environmental factors on underwater vocal communication". We inspected the first five pages of results, in addition to searching previously published review papers by the Royal Society (2005), Learmonth et al. (2006) and Schumann et al. (2013) for further references. We also used www.openknowledgemaps.org to identify further studies using the keywords, "effects of climate change on marine mammals" and "environmental conditions underwater communication". All papers obtained in our search were compiled in an Excel spreadsheet and their information is summarised (Figure 1.1) and discussed below. We treated the selected studies by year, species, research group location and visibility. We obtained a total of 136 papers and the greatest number of studies were published in 2018, followed by 2017 (Figure 1.2). A total of 20 papers did not focus on a single species, rather they included marine mammals in general. Impact factor was estimated using each journal's 2021 CiteScore, currently available on the Scopus platform. The CiteScore of a journal shows the average number of citations of its papers, thereby providing an indication of whether the publications have been considered by other researchers and is therefore, a good impact/visibility metric of scientific journals. The 2021 CiteScores ranged from 0.6 (Advances in Polar Science) to 70.2 (Nature).

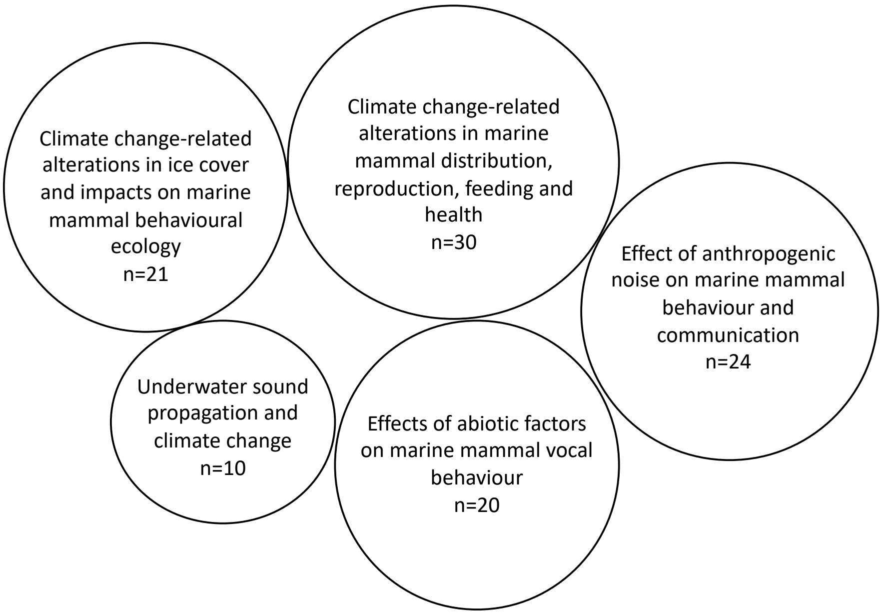


Figure 1.1. Illustration of the number of papers obtained for each topic discussed in this paper.

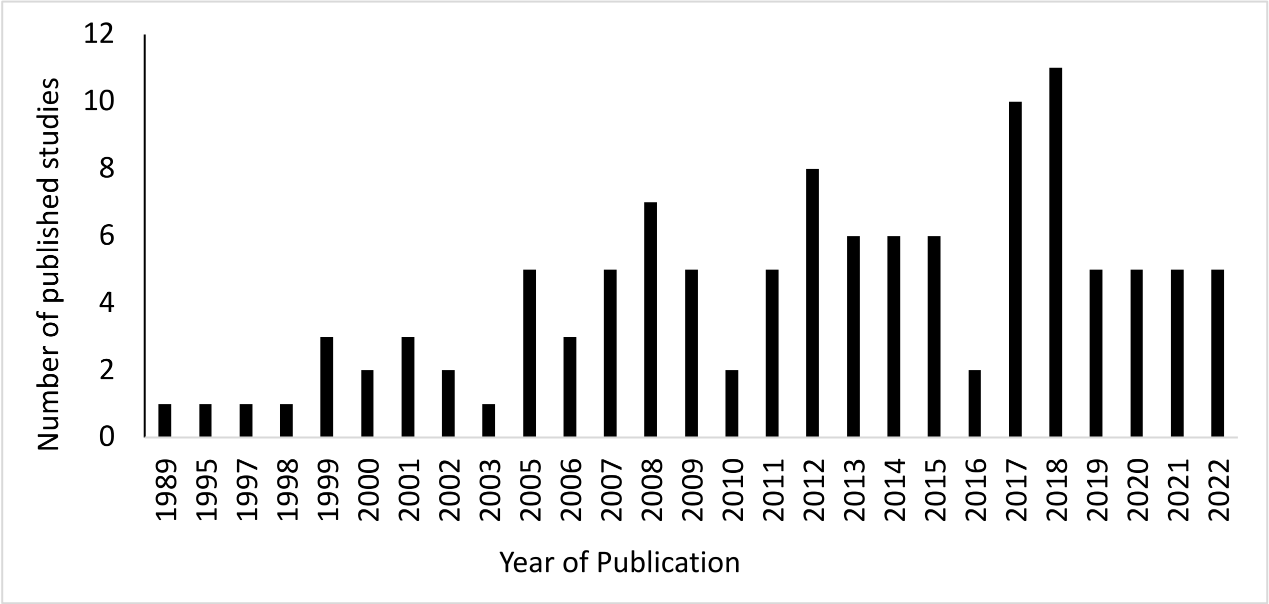


Figure 1. 2. Number and year of publication of the studies, book chapters and technical reports included in this study (n=136).

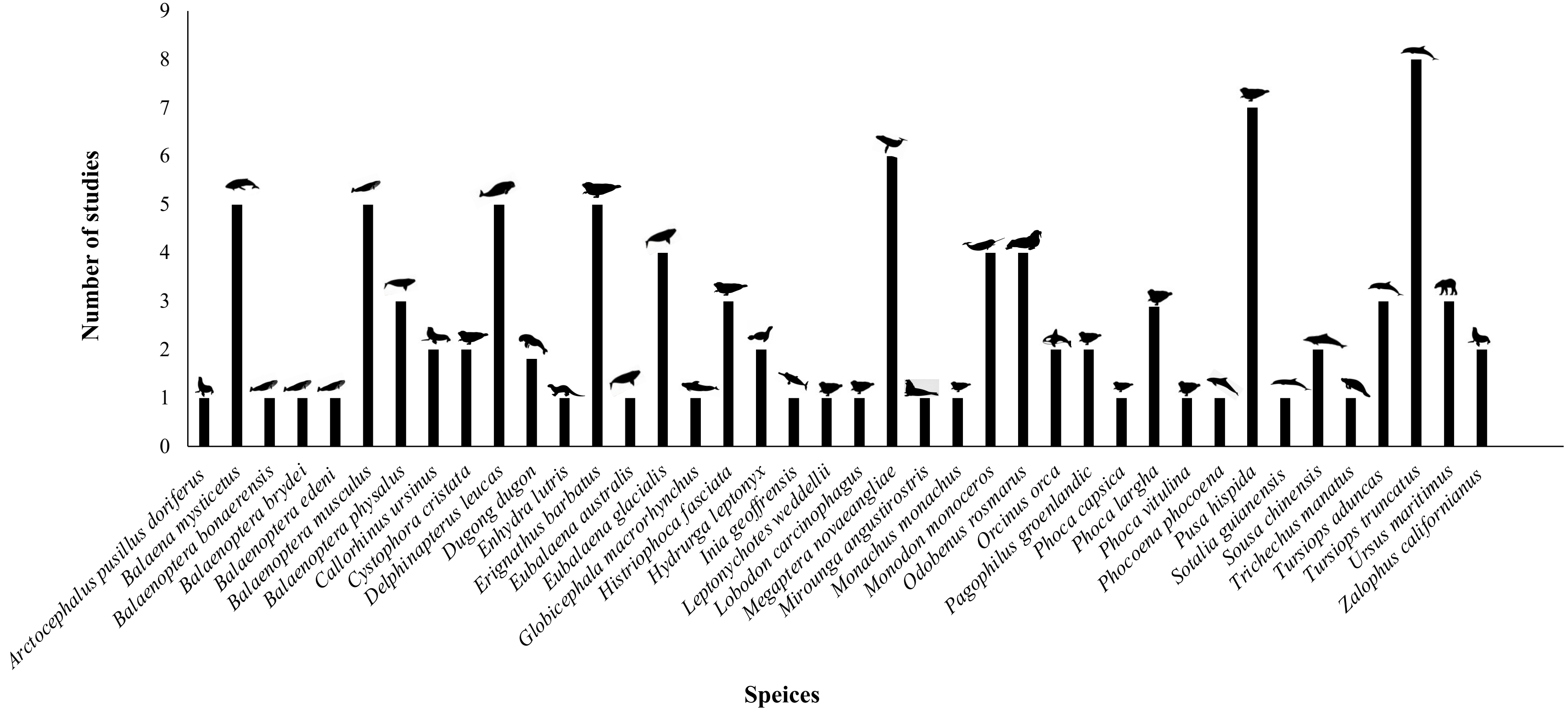


Figure 1. 3. The total number of studies on specific marine mammal species obtained in this literature review (n=41).

2.3 Results and discussion

2.3.1 Climate change and marine mammal behavioural ecology

2.3.1.1 Bibliometric review

We obtained a total of 50 papers on the topic of climate change and marine mammal behavioural ecology from our online literature search and through the review of published papers found during our literature search, that were deemed relevant to this topic i.e., papers that focused on the effects of climate change on marine mammal behavioural ecology and sound propagation. The greatest number of papers were published in 2013, followed by 2012 and 2020 (Figure 1.4). There was lower scientific interest in the years before 2006, thus this pattern may reflect the growing global concern regarding climate-induced changes in marine mammal behavioural ecology.

A total of 10 species were considered in 18 of the 50 papers (*Balaena mysticetus,* n=1; *Balaenoptera musculus*, n=2; *Delphinapterus leucas*, n=1; *Dugong dugon*, n=2; *Erignathus barbatus,* n=1; *Eubalaena glacialis*, n=2; *Hydrurga leptonyx*, n=1; *Leptonychotes weddellii*, n=1; *Lobodon carcinophagus*, n=1; *Megaptera novaeangliae*, n=2; *Monachus monachus,* n=1; *Phoca capsica,*n=1; *Pusa hispidia*, n=2; *Sousa chinensis*, n=1; *Tursiops aduncas*, n=1; *Tursiops truncatus*, n=1; *Zalophus californianus*, n=1), 15 papers considered marine mammals in general, and the remaining papers were technical reports or studies on ocean chemistry (n=17). We found that the focal species were generally either Arctic species (e.g., *Hydrurga leptonyx*, *Leptonychotes weddellii*), baleen whales (e.g., *Balaena mysticetus*, *Balaenoptera musculus*), or tropical marine mammals (e.g., *Dugong dugon, Tursiops aduncus*). Cetaceans and marine mammals that inhabit temperate waters were included in the papers that considered, “general marine mammals”. The majority of studies focused on species that occur exclusively in the Northern Hemisphere (n=8) as previously found by Orgeret et al. (2021), with seven species occurring exclusively in the Southern Hemisphere and four species occurring globally.

The CiteScores of the associated journals can be found in Table 1.1. The average 2021 CiteScore was 25.745.

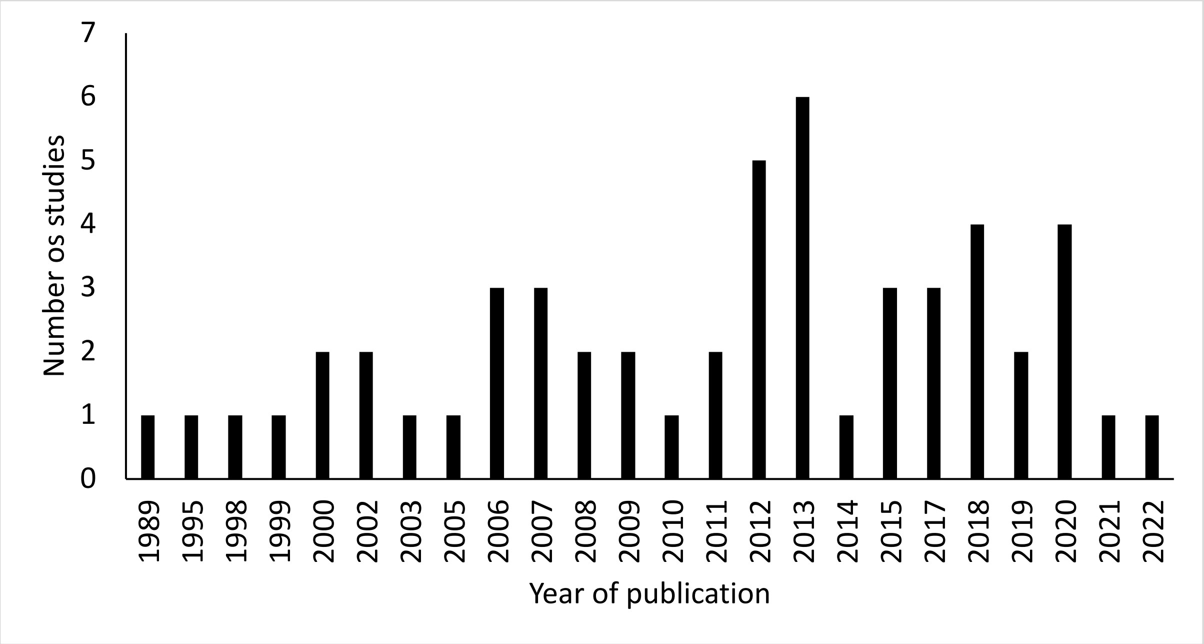


Figure 1. 4. The number of studies obtained through our literature review on the topic of the effects of climate change on marine mammal behavioural ecology (n=50).

Table 1. 1. The 2021 CiteScores for the journals of the published papers identified through our literature search. The CiteScores were not available for six journals.

Journal	**CiteScore**
Advances in Polar Science	0.6
Aus J Zoo	1.8
Aus Vet J	2.4
Biol Conserv	9.9
Can J Fish Aquat Sci	5.1
Earth's Future	11.6
Ecohealth	5.4
Ecol Appl	7.7
Ecol Evol	4.4
Ecol Model	5.3
Endang Species Res	4.4
Epidemiology & Infection	6.3
Estuar Coast Shelf Science	5.3

Front Mar Sci	5.2
Global Change Biol	17.9
Hydrol Earth Syst Sci	9.4
J Fish Disease	4.5
J Mar Biol Ass UK	3.1
Mar Freshwater Res	3.8
Mar Mamm Sci	3.6
Nature	70.2
Nature Climate Change	32.4
Nature Communications	23.2
Nature Sci Rep	6.9
PLoS One	5.6
Prog Oceanogr	7.6
Science	57.8
Vet Path	4.3
Wildl Res	3.8

2.3.1.2 Changes in ice cover and impacts on marine mammal behavioural ecology

The most apparent effects can be seen through the decrease in ice cover and, as a result, the loss of habitat for species such as polar bears, *Ursus maritimus* (Evans and Bjørge 2013). However, less ice signifies greater spatial use for some less-adapted species, such as *Megaptera novaeangliae, Balenoptera physalus* and *Orcinus orca* (Moore and Huntington, 2008; Huntington et al., 2017). Furthermore, some highly adapted ice species, such as bowhead whales, *Balaena mysticetus*, and ringed seals, *Pusa hispida*, minke whales, *Balaenoptera acutorostrata* and fin whales, *Balaenoptera physalus,* have seemingly responded positively to increasing global temperatures, due to increased ocean productivity, food resource availability and population sizes (Crawford et al., 2015; George et al., 2015; Huntington et al., 2017; Moore and Reeves, 2018).

Eleven marine mammal species depend on sea ice for their survival (Kovacs et al., 2011). It is estimated that Arctic summer ice has decreased by 2 million km^2 when comparing 1979-1988 and 2010-2019 (IPCC, 2022) and it is predicted that by 2050 the Arctic Ocean will become practically sea ice free for the first time (IPCC, 2022). Additionally, increased global temperatures have resulted in the collapse of sea-ice regimes in areas such as Svalbard, Norway, and the marine ecosystem has suffered an alteration in fish biodiversity, with a reduction in Arctic species and an increase in Atlantic fish species (Hamilton et al. 2019). Consequently, species with limited dietary plasticity such as ringed seals, have suffered a reduction in habitat range, whereas species with greater dietary plasticity such as beluga whales, *Delphinapterus leucas*, have experienced an increase their home range (Hamilton et al. 2019). A rise in global temperatures is also predicted to result in an increase of completely ice-free areas in the Antarctic, potentially providing new habitats and consequently, new dispersal and colonisation areas for native and exotic species (Lee et al. 2017). Although, this may appear to be a positive change for Antarctic biodiversity, it is still unknown whether the negative impacts, such as an increase in invasive species, will outweigh the positives impacts (Lee et al. 2017). Furthermore, suitable habitat areas for Wedell seals, *Leptonychotes weddellii,* crabeater seals, *Lobodon carcinophagus*, and leopard seals, *Hydrurga leptonyx,* were found to have decreased by 47%, due to changes in climate from the years 1979-2011 on the West Antarctic Peninsula (Forcada et al. 2012). These seal species depend on sea-ice for breeding, resting, moulting and protection from predators (Fuxing et al. 2017). Ferguson et al. (2005) found that decreased snow depth corresponded to a significant decrease in ringed seal recruitment, with less snow, shallower snow depth and warmer temperatures occurring during the period when pups are born and nursed. Furthermore, decreases in summer ice extent may result in a decrease in available food resources for Pacific walruses, *Odobenus rosmarus,* increase their predation risk and affect hunting success (Kelly, 2001; Kovacs et al., 2011). Migration timing may also be affected due to these global changes, as observed by indigenous hunters, for bearded seals, *Erignathus barbatus*, and walruses whose migration timing has begun to blend together in some regions in Alaska, resulting in their arrival at the same time in spring (Huntington et al., 2017).

2.3.1.3 Changes in distribution, reproduction, feeding and health

Davidson et al. (2011) identified 13 global hotspots of risks to marine mammal species from anthropogenic sources. They found that the risk hotspots covered only 1.7% of global oceans, however, they include, at least in part, the geographic distributions of 88 marine mammal species (Davidson et al., 2011). The distribution of some marine mammal species may be defined by their thermal tolerance (Sunday et al., 2012; Schumann et al. 2013; Hastings et al., 2020) and their responses to climate change vary considerably. MacLeod (2009) found that the ranges of 88% of cetacean species are predicted to change as a result of increasing ocean temperatures. He also found that the ranges of 50% of species, including five species of beaked whales and seven species of dolphins, were predicted to decline and the ranges of 38% of species, including three species of baleen whales and 18 species of dolphins, were expected to increase (MacLeod 2009). Thus, it can, be inferred that tropical species' ranges will extend to higher latitudes, temperate species' ranges will expand poleward, while polar species' ranges will decrease with increasing global ocean temperatures (MacLeod 2009).

The marine mammals that are most sensitive to climate change are those with marked feeding and habitat specialisations, as well as those with fragmented and reduced geographical ranges (Albouy et al., 2020). Rising sea-surface temperatures can have indirect effects on species distributions, through alterations in prey availability. For instance, blue whales, *Balaenoptera musculus*, have experienced decreases in their ranges due to the reduced availability of krill, which in turn is associated with a reduction in sea ice caused by increasing ocean temperatures (Nicol et al. 2008; Schumann et al. 2013). Furthermore, sea surface temperature and krill abundance have been found to play a key role in blue whale movement decisions during round-trip migrations (Dodson et al., 2020). Bryde's whales, *Balaenoptera brydei*, were increasingly detected in cooler waters during the period of 2000-2010, i.e., a seasonal poleward range expansion, likely driven by prey availability (Kerosky et al., 2012). Increasing temperatures may also affect predator-prey dynamics as demonstrated by Sadykova et al. (2019), who predicted a dramatic loss in predator-prey density population overlap for harbour seals and their prey (48.5% reduction) by the year 2062. Changing temperatures may also have different effects depending on sex and age class. Barbieri (2005) found that adult male bottlenose dolphins, *Tursiops truncatus*, in Florida were more likely to exploit colder waters during the summer months compared to dolphins of other sex and age classes.

Maxwell et al. (2013) found that blue whales, humpback whales, California sea lions, *Zalophus californianus* and Northern elephant seals, *Mirounga angustirostris,* had high vulnerability weights for ocean acidification and sea surface temperature anomalies in the United States. Sea surface temperature has also been found to affect the breeding success of southern right whales, *Eubalaena australis* (Leaper et al. 2006). Notably, under higher sea-surface temperatures, fewer calves were produced by whales from Argentina however, the opposite was found for whales in the Pacific Ocean, where increased sea-surface temperature resulted in a greater calf output (Leaper et al. 2006; Schumann et al. 2013). In a study by Albouy et al. (2020), the North Pacific right whale, *Eubalaena japonica*, and walrus, were found to be the most vulnerable species to ocean warming, followed by the grey whale, *Eschrichtus robustus*, the crabeater seal, the North Atlantic right whale, the dugong, *Dugong dugon* and the narwhal, *Monodon monoceros,* according to both functional and phylogenetic diversity scenarios.

Weather and climate variables play a critical role in infectious disease dynamics (Harvell et al., 2002; Sanderson and Alexander, 2020). The emergence of infectious diseases has increased significantly over the past 30 years (Harvell et al., 1999; Gulland and Hall, 2007; Simeone et al., 2015; Sanderson and Alexander, 2020) and are expected to continue to increase under current climate change predictions (Cann et al., 2013; Cai et al., 2014; Oliver et al., 2018; Sanderson and Alexander, 2020). Marine mammals are endothermic and consequently, any shifts outside their ideal temperature ranges require increased energy expenditure which may result in increased stress responses and weakened immune systems (Sanderson and Alexander, 2020). Changes in sea ice cover may affect the length of offspring suckling time, consequently impacting offspring and/or juvenile immunity (Kuiken et al., 2006). Furthermore, warmer waters can allow pathogens to expand and/or alter pathogen survival rates due to changes in salinity (Tucker et al., 2000). However, flooding may also reduce water quality and expose species to new diseases (Schumann et al. 2013). Bowater et al. (2003) found that an Indo-Pacific humpback dolphin, *Sousa chinensis*, was infected with toxoplasmosis following flood events, which was likely associated with contaminated runoff (Schumann et al. 2013). Contaminated rainfall and/or runoff-associated mortalities have also been documented for 14 humpback whales, *Megaptera novaeangliae,* 117 monk seals, *Monachus monachus* and 400 sea lions, *Zalophus californianus* (Geraci et al. 1989; Hernández et al. 1998; Scholin et al. 2000; Learmonth et al. 2006).

Sanderson and Alexander (2020) found that infectious disease mass mortalities in marine mammals were significantly impacted by season and described an upward trend in the occurrence of such events with increasing yearly global sea surface temperature anomalies. Mass mortality events caused by infectious diseases can have strong impacts on marine mammal populations, as was the case for Caspian seals (Vulnerable to Endangered, eight years after an event) and hooded seals (Lower risk/least concern to Vulnerable, two years after an event) (Sanderson and Alexander, 2020). Currently 25% of marine mammal species/sub-species that have experienced infectious disease mass mortality events are considered Endangered, 15% are Vulnerable, 5% are Near

Threatened, 10% are Data Deficient and 45% are of Least Concern by the ICUN red list (Sanderson and Alexander, 2020).

Extreme weather events are occurring more frequently, and the intensification of rainfall extremes and their increasing volume has been associated with higher temperatures as a result of climate change (IPCC, 2013; Hettiarachchi et al., 2018). Following cyclones and floods in Hervey Bay, Queensland, dugong populations were found to be significantly reduced, with an increased rate of emigration as a result of seagrass bed loss (Preen and Marsh 1995; Schumann et al. 2013). Furthermore, following Cyclone Yasi, a record number of dugong deaths was documented, which was thought to be a consequence related to reduced seagrass bed availability (Brodie and Waterhouse 2012; Schumann et al. 2013). Additionally, flood-related changes in water levels can result in alterations in abiotic conditions such as salinity and turbidity (Chen et al., 2018). Fury and Harrison (2011) found that Indo-Pacific bottlenose dolphins, *Turstops aduncus*, left estuaries and suffered mortalities following floods, as a result of reduced water salinity and/or increased turbidity (Fury and Harrison 2011; Schumann et al. 2013).

Thus, we can conclude that climate change has been documented and predicted to have severe impacts on the behavioural ecology and health of many marine species. Whether direct or indirect, many of these mammals have been and are continually negatively impacted by these anthropogenically-induced alternations in the environment and mitigation strategies must be implemented in order to conserve and maintain marine mammal species.

2.3.2 Underwater sound propagation and marine mammal vocal communication

2.3.2.1 Bibliometric review

We obtained a total of 45 studies on the topic of underwater sound propagation and marine mammal vocal communication from our literature search that were deemed relevant to this topic. Of these studies, 53.3% (n=24) focused on the impact of anthropogenic noise on marine mammal vocal behaviour, 24.4% focused on marine ocean chemistry (n=11), 13.3% seasonal/diurnal patterns (n=6), 4.4% focused on the impacts of abiotic factors on sound propagation (n=2) and 4.4% investigated the effects of abiotic factors on marine mammal vocalisation structure and production (n=2).

The greatest number of studies were published in 2017-2018, with peaks in publications throughout each decade (1997-2007; 2007-2017) (Figure 1.5). Overall, there were a greater number of published studies between 2012-2022 compared to 1997-2011, which may reflect the growing concern regarding the effects of climate change on sound propagation and marine mammal vocal behaviour.

These studies considered a total of 24 species (Table 1.2), of which 18 species were considered in papers focused on the effects of anthropogenic noise and 14 species were considered in papers focused on the effects of abiotic variables on sound propagation. The papers obtained here focused on tropical estuarine species (e.g., *Trichehus manatus, Sousa chinensis*), baleen whales (e.g., *Balaenoptera musculus, Balaenoptera physalus*), odontocetes (e.g., *Tursiops truncatus, Orcinus orca*) and pinnipeds (e.g., *Monodon monoceros, Erignathus barbatus*).

The 2021 CiteScores for each journal are shown in Table 1.3. The average CiteScore was 7.555.

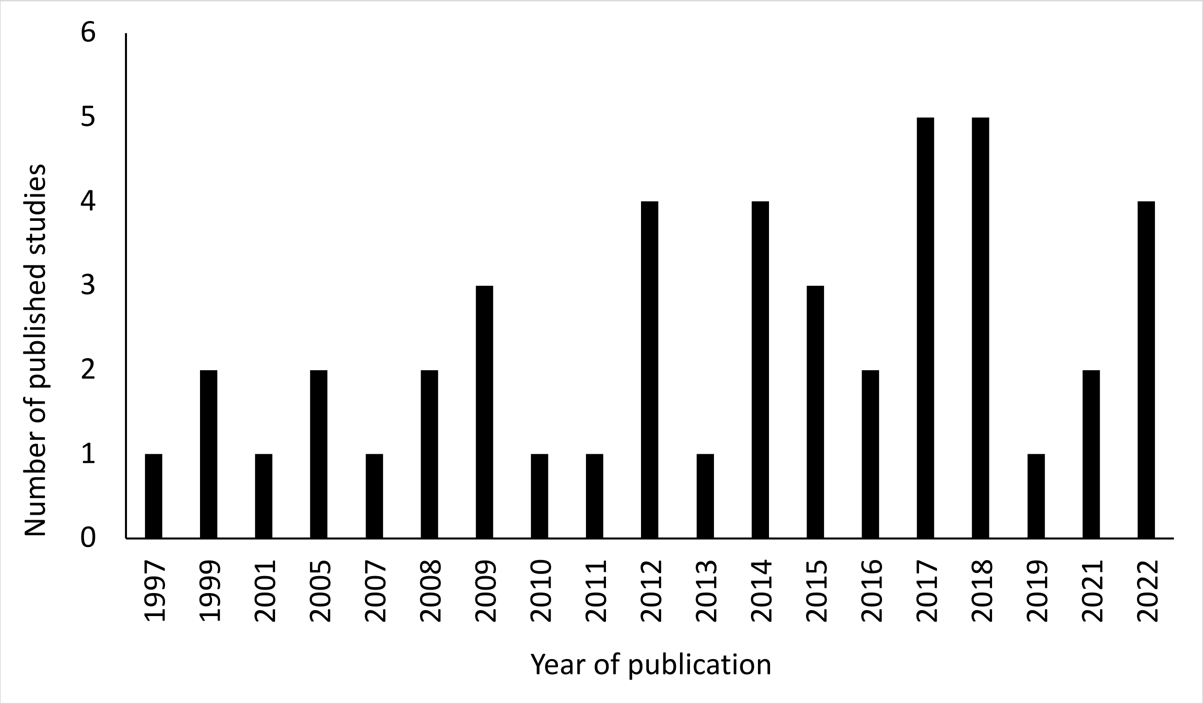


Figure 1. 5. Representation of the publication year of the scientific papers obtained through our literature search.

Table 1. 2. List of species identified in the published papers focused on the effects of abiotic variables on marine mammal vocal behaviour, obtained through our literature search.

Species	Number of studies	Reference
Arctocephalus pusillus doriferus	1	Tripovich et al., 2012
Balaena mysticetus	2	Stafford et al., 2018
Balaenoptera bonaerensis	1	Menze et al., 2017.
Balaenoptera edeni	1	Putland et al., 2017.
Balaenoptera musculus	3	Menze et al., 2017; Melcón et al., 2012; Shabangu et al., 2017.
Balaenoptera physalus	3	Menze et al., 2017; Cholewiak et al., 2018; Williams et al., 2014.
Delphinapterus leucas	2	Lesage et al., 1999; Stafford et al., 2018.
Erignathus barbatus	2	de Vincenzi et al., 2019; Marcouz et al., 2017.
Eubalaena glacialis	5	Peng et al. 2015; Parks et al., 2011; Rolland et al., 2012; Rice et al., 2014; Affatati et al. 2022.
Globicephala macrorhynchus	1	Jense et al., 2009
Hydruga leptonyx	1	Menze et al., 2017.
Inia geoffrensis	1	Amorim et al., 2016.
Megaptera novaeangliae	4	Kowarski et al., 2018; Mercado and Frazer, 1999; Cholewiak et al.,

		2018; Williams et al., 2014.
Mirounga angustirostris	1	Kastak et al., 2005
Monodon monoceros	1	Marcoux et al., 2017
Odobenus romsarus	1	Marcoux et al., 2017.
Orcinus orca	2	Williams et al., 2014; Thornton et al., 2022.
Phoca vitulina	1	Kastak et al., 2005
Sotalia guianensis	1	Deconto et al., 2021.
Sousa chinensis	1	Wang et al., 2015.
Trichechus manatus	1	Miksis-Olds et al., 2007
Tursiops aduncus	1	Morisaka et al., 2005
Tursiops truncatus	6	Scarpaci et al., 2001; Jensen et al., 2009; Guerra et al., 2014; Fouda et al., 2018; Pine et al., 2021; Gospić and Picciulin, 2016.
Zalophus californicus	1	Kastak et al., 2005

Table 1. 3. The journals of the published papers found during our literature search. CiteScores were not available for five journals.

Journal	**CiteScore**
Earth's Future	11.6
J Acoust Soc Am	3.7
Geophys Res Lett	8.5
Endang Species Res	4.4
Polar Biol	4
Bioacoustics	3.6
Biol Lett	6.2
Mar Poll Bull	9.2

Endang Species Res	4.4
Geophys Res Lett	8.5
Mar Ecol Prog Ser	5.3
Nature Geoscience	28.3
Mar Ecol Prog Ser	5.3
J Acoust Soc Am	3.7
Acoustics Today	NA
Bioacoustics	3.6
Mar Mam Sci	3.6
Oceanography	6.5
Polar Biol	4
PLoS ONE	5.6
R Soc Open Sci	5.7
J Acoust Soc Am	3.7
Mar Mam Sci	3.6
J Mamm	4.5
Biol Lett	6.2
Int J Environ Res Pub Health	4.5
Global Change Biol	17.9
Ecol Inform	5.4
Proc Roy Soc B	8.3
J Cet Res Manage	2
PLoS ONE	5.6
Mar Tech Soc J	1.1
Animal Conservation	5.9

2.3.2.2 Marine mammal communication and the influence of environmental factors on sound transmission

Environmental stability has been shown to impact calling activity, as documented in bearded seals, *Erignathus barbatus* (De Vicenzi et al., 2019) and Antarctic blue whales in the Southern Ocean, where specific call rates were found to be higher under different environmental temperatures (Shabangu et al., 2017). This suggests that species may be vulnerable to alterations in sea surface temperatures caused by climate change and could result in habitat loss, distributional shifts, as well as impacting the recovery of species (Shabangu et al., 2017). Furthermore, the detection of marine mammal sounds in the Arctic has been found to be strongly influenced by ice cover, where narwhals and bowhead whales were only detected before ice formation and bearded seals and walruses were only detected after ice formation (Marcoux et al., 2017). As such, alterations in sea ice caused by anthropogenically-induced climate change, may also affect marine mammal vocal behaviour and, consequently, their behaviour ecology. Additionally, tidal phase, time of day, water temperature at 300m and air temperature have all been found to influence narwhal click detection (Marcoux et al., 2017). Humpback whale singing was also found to be correlated with day length, sea surface temperature and latitude (Kowarski et al., 2022).

Deconto et al. (2021) found that dissolved oxygen and turbidity significantly explained variations in the propagation of Guiana dolphin, *Sotalia guianensis*, sounds where relative amplitude decayed with lower quantities of dissolved oxygen and greater water turbidity. However, temperature and salinity were not found to be important in sound variation (Deconto et al., 2021). This study also demonstrated that the creation of the Valo Grande Channel resulted in a 37% reduction in dolphin whistle amplitude, due to the high influx of sediment and consequent increase in turbidity. Amorim et al. (2016) found that frequency and time parameters of Amazon River dolphin, *Inia geoffrensis*, vocalisations differed according to water turbidity. In conditions with higher quantities of suspended particles, scattering processes as well as attenuation and geometric spreading are greater (Amorim et al., 2016). In the case of the Amazon River dolphin, these conditions resulted in lower maximum and centre frequencies, bandwidths and number of harmonics (Amorim et al., 2016). To our knowledge these are two of the only studies investigating the effects of turbidity on marine mammal vocalisation structure, reflecting the significant gap in knowledge on this topic of research. As such, more studies are required in order to implement mitigating strategies, since the predicted increase in storm frequency may result in increases in the amount of sediment particles, particularly, in coastal and estuarine marine environments (Chen et al., 2018).

2.4 Conclusions

We found that research papers focused on the effects of climate change on marine mammal behavioural ecology were published in journals with a much greater scientific outreach, compared to the research papers focused on the effects of environmental factors on marine mammal vocal behaviour. This finding reflects the distinct gap in knowledge on the potential, and reported, impacts of abiotic factors on marine mammal communication, which is alarming considering the current global situation. However, we did observe that the few existing studies on this topic were published in recent years, which may indicate that this is becoming a recent area of interest in the scientific community.

Increasing temperatures, in particular, have been shown to strongly impact marine mammal behaviours, influencing not only feeding and reproductive behaviours but also distribution patterns. However, it is important to investigate the current and predicted impacts of climate change on marine mammal acoustic behaviour, since many behaviours (e.g., feeding, reproductive, migratory) are associated with vocal patterns.

3 Article 1 - Vocal complexity in Antillean manatees (Trichechus manatus manatus)

3.1 Introduction

Complexity in vocal behaviour can be expressed in the size of a vocal repertoire, the combination of different vocalisation types (Crockford & Boesch, 2003; Ouattara et al., 2009; Krams et al., 2012; Bouchet et al., 2013), the alteration of their physical structure (Mitani & Brandt, 1994; Slocombe et al., 2009), their use in different behavioural or ecological contexts and by different individuals (Gustison et al., 2012; Krams et al., 2012; Gustison & Bergman, 2016; Cheney & Seyfarth, 2018). The ability of animals to modify the structure and production of their calls may influence the success of their survival. For instance, an individual's ability to modify their vocalisations in differing natural environmental scenarios (e.g., fluctuating water turbidity, pH and tide regimes) and anthropogenically-induced scenarios (e.g., presence of anthropogenic noise), could optimise intraspecific communication - particularly between mothers and calves and between mating individuals – by improving call propagation and delivery (Okabe et al., 2012; Vasconcelos et al., 2012; Caorsi et al., 2017; Charlton et al., 2018; Nunez & Rubenstein 2020). Despite potentially optimizing communication, the ability of animals to modify their vocalisations may involve behavioural and ecological costs. Altering vocalisations can have metabolic costs that can vary at an individual and species level (Ophir et al., 2010; Noren et al., 2013; Holt et al., 2013; Dechmann et al., 2013 Holt et al., 2015). For instance, oxygen consumption increases during call production (Bucher et al., 1982; Ophir et al, 2010; Holt et al., 2015; Holt et al., 2016; Chaverri et al., 2021), which can ultimately affect an animal's growth, reproduction, and anti-predation strategies (Brown et al., 2004).

The production of different call types and differences in call structure have been observed for various marine mammals. For instance, cetaceans use different types of vocalisations in different behavioural scenarios i.e., they produce echolocation clicks when foraging and whistles for communicating with conspecifics (Tyack, 1986; De Souto et al., 2012). Sperm whales, *Physeter microcephalus,* produce clicks with different structures, where longer clicks, lower click repetition rates, and intensity peaks at 1.8 and 2.8 kHz correlate with the presence of mature males (Weilgart & Whitehead, 1988). Captive Antillean manatees, *Trichechus manatus manatus*, produce vocalisations that differ in call length between age groups and demonstrate differences in fundamental frequency parameters between sexes (Sousa-Lima et al., 2008; Umeed et al., 2018). Furthermore, some marine mammal species produce signature vocalisations such as bottlenose dolphins, *Tursiops truncatus*, which produce whistles that have distinctive structural characteristics, indicating the production and use of individual signature whistles (Caldwell & Caldwell, 1965; Caldwell & Caldwell, 1979). Additionally, Amazonian manatees, *Trichechus inunguis*, produce individually stereotyped vocalizations, believed to contain individual signature information (Sousa-Lima et al., 2002) and captive Antillean manatees produce isolation calls thought to contain individual identity information (Sousa-Lima et al. 2008).

Sex and individual-related variability in vocalisations are widespread across social animals and has been linked with social complexity (Tibett & Dale, 2007; Kram et al., 2012). Across marine mammal species, where parental care is involved, the parent that invests the most care will produce more vocal signals, often up to ten times their average vocalisation rate (Fripp & Tyack, 2008; Dunn et al., 2017). Usually, there are specific vocalisations associated with parental care (Marcoux et al., 2006). For many marine mammal species, including manatees (Sousa-Lima et al., 2002; Sousa-Lima et al., 2008), dugongs, *Dugong dugon* (Fuentes et al., 2016), cetaceans (Rendell et al., 2019) and pinnipeds (Boness & Bowen, 1996), mothers are primarily responsible for the survival of offspring, including gestation, lactation, weaning and post-weaning care (Rendell et al., 2019). Therefore, one could expect that females responsible for parental care would produce vocal repertoires constructed of high vocal signal repetitions of a few base vocalisation types, i.e., simple and repetitive repertoires. Narwhal mothers, *Monodon monoceros*, for instance, produce highly stereotyped and individually specific contact calls when separated from their calves, which are thought to contain identity information (Ames et al., 2021). Atlantic walrus, *Odobensu rosmarus rosmarus*, barks propagate at greater distances over water with certain acoustic features being highly resistant to degradation, suggesting that these features may be key for individual identification (Charrier et al, 2010). For captive belugas, *Delphinapterus leucas,* individual differences have been found for pulse patterning, suggesting the use of these calls for individual recognition (Morisaka et al., 2013). On the other hand, Indeck (2020) found that mother and calf humpback whales, *Megaptera novaeangliae,* produce vocalisations that are more difficult to detect - i.e., they have lower call rates with reduced acoustic level and limited active space, and they can also modify their behaviour to avoid unwanted interactions and to maintain acoustic contact.

Captive Antillean manatees produce single-note vocalisations often with multiple harmonics, with the fundamental frequencies falling between 0.64 to 5.23 kHz (Umeed et al., 2018; Merchan et al., 2019) with maximum frequencies potentially reaching up to 150kHz (Ramos et al., 2020). The fundamental frequencies, contour and frequency range of captive Antillean manatee vocalisations vary significantly between individuals, sex and age groups (O'Shea & Poché, 2006; Sousa-Lima et al., 2008; Umeed et al., 2018; Merchan et al., 2019). Umeed et al. (2018) and Sousa-Lima et al. (2008) demonstrated that captive adult females produced vocalisations with longer mean durations and lower mean maximum frequencies compared to adult males. Furthermore, studies have demonstrated that captive juvenile male Antillean manatees produce vocalisations with higher mean maximum frequencies compared to captive adult manatees (Umeed et al., 2018) and that calves produce vocalisations with higher frequency parameters compared to adult manatees (Sousa-Lima et al., 2008). Antillean manatees can spend time solitarily, as mother-calf pairs, mating pairs or as small and large groups (Alves et al., 2013; von Fersen and Walb 2018; Favero eta l., 2020; Gomez-Carrasco et al., 2020). Therefore, the possibility of having different group dynamics in the wild (and captivity), may suggest some level of vocal complexity in the species in order to coordinate communication effectively.

In the present study, we aimed to assess vocal complexity in Antillean manatees by evaluating the variation in the structure and production of vocalisations of animals living in captivity (i.e., artificial pools) and in reintroduction enclosures (i.e., readaptation enclosures in a natural estuarine area) in North-eastern Brazil. Here we aimed to i) categorise calls for individuals living in captive and natural reintroduction enclosures to assess whether variants of each call category exist; ii) investigate whether call production (i.e., call type, call rate, and overall call and behavioural patterns) varied between the study groups, and ii) investigate whether individuality could be identified when analysing the structure of Squeak vocalisations. We tested the following predictions: a) Female manatees produce a higher number of vocalisations with a more stereotypical repertoire, i.e., a repertoire composed of a limited number of call types, produced repetitively due to the presence of related individuals in the captive pool; b) The number of vocalisations differs depending on social context - the greater the number of animals in an enclosure (i.e., the more complex social scenario in the present study) the greater the number of vocalisations (call rate) produced (i.e., the more complex the vocal behaviour); c) the structure of Antillean manatee vocalisations differs between individuals. This study will improve our understanding of whether Antillean manatees conform to the predictions of the social complexity hypothesis for communication (i.e., "social unities that occur at high densities results in individuals that interact with one another at higher rates" - see Freeberg et al., 2012 for a review).

3.2 Material and methods

3.2.1 Study sites and animals

We investigated captive Antillean manatees housed at the Instituto Chico Mendes de Conservação da Biodiversidade/ Centro Nacional de Pesquisa e Conservação da Biodiversidade de Mamíferos Aquáticos (ICMBio/CMA) on Itamaracá Island, Pernambuco, Brazil (Table 2.1, Figure 2.1). We also investigated manatees in the reintroduction centre in the Área de Proteção Ambiental (APA) Costa dos Corais/ICMBio, Porto de Pedras, Alagoas, Brazil (Table 2.1, Figure 2.1).

This study was comprised of two parts. Firstly, we investigated the overall call structure and production in four manatee study groups. We then specifically investigated the production of signature vocalisations through the analysis of Squeak vocalisations (see call description below) from four individually recorded manatees. The recording and observation methods for each part of the study are detailed below.

Table 2. 1 Characteristics of the studied Antillean manatees. Estimated ages of each manatee housed in captive or reintroduction conditions (reintroduction enclosure 1 or 2) that were included in the study at the time of data collection in 2019; weight and length measurements were obtained from the closest month (January-June 2019) before or after the study was carried out. The ages were estimated by the staff at the ICMBio/CMA based on the date of birth (for the manatees that were born in captivity i.e., Bela, Carla, Sheila) or based on the physical condition (size, appearance) of manatees at the date of rescue (for the animals that were stranded and subsequently rescued). For the latter, the estimate age at time of rescue until the date of the study recordings was used. PE: State of Pernambuco, Brazil; AL: State of Alagoas, Brazil. *Asterisks indicate an extensive time-lapse (>6 months) between the measurements and the study period.

Individual	Sex	Estimated age (yr.)	Age Category	Weight (kg)	Length (cm)	Housing Condition	Location
Bela	Female	8	Adult	585	287	Captive	PE
Canoa	Female	14	Adult	358*	271*	Captive	PE
Carla	Female	22	Adult	554	295	Captive	PE
Paty	Female	4	Juvenile	310	248	Captive and Reintroduction 2	PE
Sheila	Female	22	Adult	572	307	Captive	PE
Xuxa	Female	32	Adult	828	305	Captive	PE
Daniel	Male	9	Adult	356	275	Captive	PE
Parajuru	Male	6	Adult	325	251	Captive	PE
Poque	Male	27	Adult	387	256	Captive	PE
Zoé	Male	14	Adult	290	251	Captive	PE
Assu	Male	20	Adult	392*	280*	Reintroduction 1	AL
Netuno	Male	28	Adult	476*	315*	Reintroduction 1	AL
Aratí	Male	9	Adult	301	263.5	Reintroduction 2	AL

Raimundo	Male	8	Adult	350	277	Reintroduction 2	AL

3.2.2 Observations and recordings – Part 1

When investigating call structure and production, between February 2019 and May 2019, we observed and recorded six captive females (range: 4 - 32 years old); four captive males (range: 6 - 27 years old) and four males in two reintroduction enclosures (range: 8 - 28 years old) (Table 2.1).

The six captive females were all kept in the same pool with dimensions of 10 m x 5 m, and 4 m deep (Figure 3.1-1a) The four captive males were kept in the same pool with dimensions of 8m x 5m and 4m deep (Figure 2.1 a, b).

The males in the reintroduction enclosures were kept in two separate enclosures. Assu and Netuno were the older males kept in reintroduction enclosure 1, which measured 27 m x 13 m. Aratí and Raimundo were the younger males kept in reintroduction enclosure 2, which measured 31m x 17m. The depths of the reintroduction enclosures varied with tide regimes as the enclosures are situated in the mangroves of the Tatuamunha River. The reintroduction enclosure walls are constructed from wooden poles with spaces between each pole (Figure 2.1 c, d). Therefore, the manatees in the reintroduction enclosures experienced changes in tide level, water temperature and turbidity. The average daily water temperature during our recordings ranged from 29.2 °C - 32.2 °C and the average daily water pH ranged from 6.83 – 7.78.

We recorded the animals housed in captive and reintroduction enclosures during daylight hours, from 7 am until 5 pm between February 2019 and May 2019. We recorded the vocalisations of the animals during focal group recording sessions of 10-min duration. We did not isolate the animals during these recordings. Thus, the call counts used to calculate call proportions and rates were obtained for each social group (i.e., captive males, captive females, males in reintroduction enclosures 1 and 2) and not individual animals. We conducted a total of 217 focal group sessions (36.3 hr total), where 3701 vocalisations were recorded and considered in our analysis. We conducted 74 focal sessions in the captive female enclosure (12.7 hr of recordings), 47 focal group sessions in the captive male enclosure (7.8 hr of recordings) and 42 (7 hr of recordings) and 53 (8.8 hr of recordings) focal group sessions in reintroduction enclosures 1 and 2, respectively. We calculated the proportion (%) of each behaviour produced by the study animals during these observations so that we could compare the behavioural patterns of the study groups and relate them to call patterns.

We recorded the Antillean manatee vocalisations using a Wildlife Acoustics SM4 recorder (linear frequency response 20Hz-48kHz) connected to an SM3 Hydrophonex hydrophone, which was placed inside a pipe, following the methodology described by Umeed et al. (2018) in order to protect the hydrophone from the manatees. Previous tests performed by Umeed et al. (2018) showed no evidence of echoes or reflection of the vocalisations recorded with the captive manatees, however, we acknowledge that the concrete material used to construct the oceanariums may have affected the sound propagation of the recorded vocalisations.

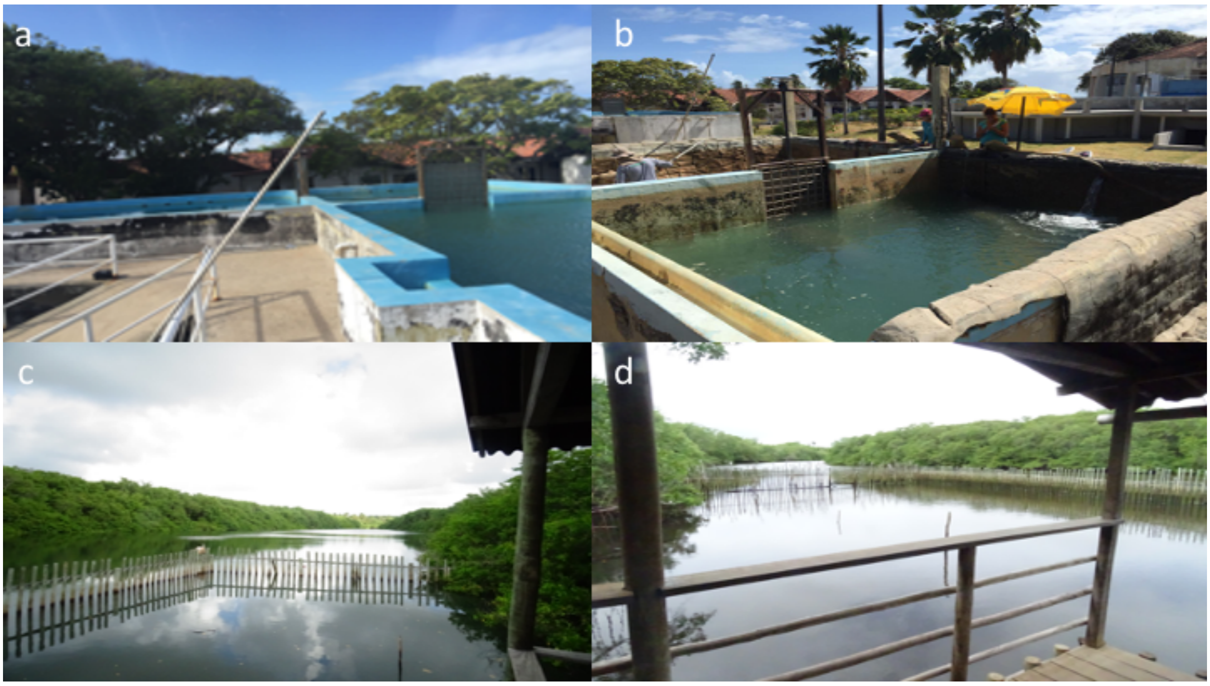


Figure 2. 1. Images of the a) captive female pool, b) captive male pool, c) semi-captive male enclosure 1 and d) semi-captive male enclosure 2. Photo sources: RU, KL.

3.2.3 Observations and recordings – Part 2

To assess whether Antillean manatee call structure reveals caller identity and/or suggests the production of signature vocalisations, we individually recorded four manatees for approximately 30 min each, using a Cetacean Research SQ26-H1 hydrophone, connected to a Zoom H1 recorder (linear frequency response 20Hz-20kHz). The recordings were taken in the reintroduction enclosures at the APA Costa dos Corais/ICMBio, Porto de Pedras, Alagoas, during a routine veterinary health check-up from 30 November to 2 December 2020. During the health check-up, the veterinarians removed each animal from the pool at low tide. This allowed us to record the remaining animal in its respective pool, individually. We observed the animals *Ad libitum* while the vocalisations were recorded. It is important to note that the recorded manatees may have been able to hear free-living manatees in the river and the other manatees in the nearby reintroduction enclosure. We recorded one juvenile female called Paty (an individual previously included in the captive female recordings in part 1 of the present study), and three adult males called Raimundo, Assu and Netuno (individuals from the reintroduction enclosures also previously included in part 1 of this study). Paty was transferred from the captive enclosure in Pernambuco (ICMBIO/CMA) to reintroduction enclosure 2 in Alagoas (APA Costa dos Corais) in July 2019. Paty and Raimundo inhabited reintroduction enclosure 2, and Assu and Netuno were housed in reintroduction enclosure 1 during the recording period.

A period of 10 min was allocated before recording each animal separately, to allow the individual to resume normal behaviours following the removal of their conspecific from the enclosure. Recordings were conducted at a maximum distance of 5m from the animals at low tide. In these enclosures water depth varies from 3.5 m to 1 m, depending on the tide. We recorded the animals with the help of a kayak on the water or from a platform, which provides access to the reintroduction enclosures. We selected the Squeak call type (Figure 2.2) as it was concurrent with the isolation vocalisations described by Sousa-Lima et al. (2008) for captive Antillean manatees. It was also the most tonal call type observed in our study, which is described in the literature as being a common isolation call in mammals (Zeifman, 2001; Newman, 2004; Newman ,2007; Lingle et al., 2012). Only Assu produced one other vocalisation type (Trill variant 2, see Figure 2.3), thus we only included Squeak calls in our analyses.

3.2.4 Acoustic analysis and call categorisation

We generated spectrograms to analyse the vocalisations using the Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33ms, 50% overlap).

We calculated the call rates for each group of recorded animals (i.e., captive females, captive males, males in reintroduction enclosure 1 and males in reintroduction enclosure 2). Call rates were calculated by dividing the total number of calls by the number of animals in each group and then per focal recording session, in order to compare call rates between groups. Additionally, we calculated the proportion (%) of each call type produced by the study animals.

We also obtained the structural variables of the calls using spectrograms generated in Kaleidoscope Pro 5. We measured 11 different structural variables from the vocalisations (Tables 2.2, 2.3, 2.4).

Table 2. 2. Definition of acoustic variables used in our analysis. The measurement description followed Umeed et al. (2018). All measurements were taken from the fundamental harmonic from spectrograms generated using Kaleidoscope Pro5 software, Wildlife Acoustics.

Structural variable	**Measurement description**
Duration (ms)	Time from the start of the vocalisation to the end of the vocalisation
Frequency of maximum energy (kHz)	Frequency at which the greatest amount of energy is found, derived from the power spectra.
Maximum and minimum frequency (kHz)	Maximum and minimum frequency of the call
Start and end frequency (kHz)	Start and end frequency of the call
Bandwidth (kHz)	Maximum minus minimum frequency
Distance from start to maximum frequency (ms)	Time from the start of the call to the point of the maximum frequency obtained
Distance from maximum frequency to the end of the call (ms)	Time from the point of maximum frequency to the end of the call
Number of harmonics	The total number of harmonics observed for each call, including subharmonics.
Inter-harmonic frequency interval (kHz)	The minimum frequency of the second harmonic minus the maximum frequency of the fundamental harmonic

Calls were initially classified subjectively into four categories: Squeaks, Trills, Chirps and Pulse calls (Table 2.3) and their variants. One single researcher (RU) categorized the calls. Call categorisation was based on visual spectrogram inspections and by using previously published descriptions of manatee calls. Variants of each call category were defined here as vocalisations that met the overall visual criteria for inclusion in the main call category but demonstrated specific and consistent subtly varying physical structures resulting in their classification as different variants of the main call category.

Table 2. 3.Subjective classification of call categories.

Call Category	**Description**
Squeaks	Squeaks had a harmonic structure and were defined following Umeed et al. (2018) and Brady et al. (2020) where we grouped "Squeaks, High Squeaks, Squeak-Squeals and Squeals" defined by Brady et al. (2020) into a single category of "Squeaks".
Trills	The Trill category was adapted following Umeed et al.'s (2018) classification, where we categorised trills, screeches and creaks into a single category i.e., noisy calls with some degree of deterministic chaos, often with the absence of a harmonic structure and a predominance of low frequency energy (Buder et al., 2008).
Chirps	Chirps were classified as short harmonic vocalisations following the same classification as Brady et al. (2020) in order to aid potential comparisons.
Pulse calls	Pulse calls often demonstrated a "cross-hatching" pattern during the pulse phonation, with the distance between harmonics approaching the effective frequency resolution of the spectrogram (based on Buder et al., 2008 descriptions, see Figure 3-3.1-5).

3.2.5 Statistical Analysis

3.2.5.1 Call categories and variants

We performed a discriminant function analysis (DFA) using the leave-one-out cross validation to confirm if our subjective classification of call categories could objectively separate the calls into the Squeak, Trill and Chirp categories. We used the software SPSS v 28 (IBM Statistics) to perform the DFAs in this study. We included four variables in the analyses: duration (ms), frequency of maximum duration (kHz), maximum frequency (kHz) and the number of harmonics. These four variables were selected due to their inclusion in previous studies investigating the vocal structure of manatee vocalisations (Sousa-Lima et al. 2008; Umeed et al. 2018). Pulse calls were not included in the DFA due to the small sample size (n=8) and the fact that only two variables (duration (ms) and frequency of maximum energy (kHz)) were analysed for this call type. Trill variant 4 was also excluded from the DFA analysis, as this call presented a greater level of chaos and no defined harmonics. A Permutational Multivariate ANOVA (PERMANOVA), using Primer 6 software, was then conducted to test the statistical validity of the subjective groups that we considered in the DFA, in order to determine the accuracy of call categorisation. The same analysis sequence (i.e., DFA followed by PERMANOVA) was performed to test for the differences between the variants of each call category. Whenever possible, we considered 10 sample vocalisations from each animal group (i.e., captive females, captive males, reintroduction males 1, and reintroduction males 2) for each call category variant. Thus, at least four different individuals contributed to the final sample in the DFA, however it is likely that more individuals contributed due to the number of individuals in each enclosure. Ten vocalisations were selected as this was the minimum number of calls produced for each variant, with the exception of Pulse calls.

3.2.5.2 Call production/usage

We performed a Permutational ANOVA to test for differences in the call rates produced during the focal group sessions and to evaluate differences in call production between the study groups. We used chi-square tests with contingency tables to compare both the vocal and the behavioural patterns of the study groups, to evaluate differences in call usage.

3.2.5.3 Individuality in call structure

To determine if there was individuality in the structure of Squeak calls, we performed a Discriminant Function Analysis (DFA) using the leave-one-out cross validation to compare the physical structures of the vocalisations produced by the four individually recorded animals. We included 10 vocalisation samples from each animal and four acoustic variables in this DFA (i.e., duration, frequency of maximum energy, maximum frequency and number of harmonics). A total of 10 vocalisations were selected as this was the minimum number produced by all individuals. Additionally, we performed a PERMANOVA and a PERMDISP analysis to test for the homogeneity of dispersions using Primer 6 software. We used this sequence of analysis (DFA / PERMANOVA / PERMDISP) as the DFA is a multivariate statistical technique used to analyse (eventually weighing) variables that distinguish particular groups. This analysis identifies which variables discriminate between categorical groups however, it does not statistically evaluate which categorical groups are significantly different among them. The latter analysis was performed here using a PERMANOVA (i.e., to verify whether the Squeak calls of the individual manatees differed statistically). The PERMDISP analysis was used here to confirm that the differences between groups were related to their position in multivariate space and not to dispersion (thus, PERMDISP tested for multivariate homocedasticity).

3.3 Results

3.3.1 Call categories

We subjectively grouped vocalisations into four categories: Squeak, Trill, Chirp and Pulse calls (Table 2.3). Statistical analyses comparing the physical structure (Table 2.4) of Squeak, Trill and Chirp categories confirmed our subjective categories. We found that all variables used in the DFA contributed to differentiate Squeak, Trill and Chirp call categories (Duration: Wilks' Lambda=0.769; F= 39.857; df1=2; df2= 265 p <0.001; Frequency of maximum energy: Wilks' Lambda= 0.970 ; F= 4.115; df1= 2; df2= 265 p = 0.017; Maximum frequency: Wilks' Lambda= 0.845; F=24.371 df1= 2; df2= 265; p <0.001; Number of harmonics: Wilks' Lambda=0.793; F= 34.539; df1=2; df2= 265; p <0.001). Function 1 explained 51.7% of the variance and Function 2 explained 48.3%. We correctly classified 66.4% of the calls that we subjectively assigned to the different call categories, using a leave-one-out cross-validated DFA. The PERMANOVA tested the statistical validity of the subjective groups that we considered to perform the DFA and confirmed the accuracy of our call categorisation (df = 2, SS = 166.47, MS = 83.237, Pseudo-F = 24.467, p (perm)=0.0001; Unique permutations= 9944). Pair-wise tests confirmed that Squeaks were different from Trills (t= 5.2928; p(perm)= 0.0001; Unique permutations= 9943), Squeaks were different from Chirps (t= 4.3616; p(perm)= 0.0001; Unique permutations= 9942;), and Trills were different from Chirps (t= 5.285; p(perm)= 0.0001; Unique permutations= 9960).

Table 2. 4. Structure of Antillean manatee calls and their variants for all the recorded study manatees. Average values with standard error of the 11 call variables analysed. Frequency measurements in kHz and time measurements in ms. NA = not applicable.

Vocal signal	**Syllable Duration(ms)**	**Frequency of maximum energy (kHz)**	**Maximum Frequency (kHz)**	**Minimum Frequency (kHz)**	**Bandwidth (kHz)**	**Start frequency (kHz)**	**End frequency (kHz)**	**Duration from start to maximum frequency (ms)**	**Duration from maximum frequency to end (ms)**	**Number of harmonics**	**Frequency interval between harmonics (kHz)**
Squeak 1(n=40)	279.67±12.31	3.18±0.1	3.68±0.1	3.17±0.1	0.51±0.07	3.44±0.11	3.38±0.11	61.75±12.35	217.91±13.28	3.73±0.50	1.40±0.13
Squeak 2(n=40)	273.34±20.25	3.16±0.11	3.83±0.10	2.94±0.08	0.88±0.07	3.21±0.10	3.31±0.12	44.37±7.24	228.97±20.76	5.50±0.60	1.85±0.15
Squeak 3(n=30)	268.94±12.23	0.98±0.13	1.29±0.13	1.09±0.13	0.20±0.02	1.20±0.14	1.14±0.13	59.41±9.12	209.53±16.24	11.03±0.81	0.48±0.06
Squeak 4(n=20)	261.35±7.13	3.81±0.09	4.92±0.20	3.71±0.12	1.21±0.25	4.64±0.14	4.07±0.14	33.20±12.46	228.15±11.71	3.35±0.51	1.91±0.22
Squeak 5(n=10)	271.79±15.71	2.17±0.10	2.58±0.10	2.21±0.09	0.37±0.04	2.37±0.10	2.29±0.09	77.79±7.69	194.00±15.15	11.68±0.65	1.00±0.12
Chirp (n=30)	147.46±7.89	2.76±0.20	3.46±0.24	2.74±0.20	0.72±0.07	3.02±0.25	3.18±0.21	31.15±7.55	116.31±10.78	3.33±0.44	0.72±0.72
Trill 1(n=18)	249.75±9.53	3.45±0.10	4.44±0.14	3.50±0.16	0.94±0.20	4.20±0.13	3.90±0.14	38.60±13.27	211.15±17.78	2.44±0.27	1.96±0.22
Trill 2(n=40)	308.22±13.20	2.86±0.13	3.66±0.15	2.93±0.12	0.72±0.07	3.36±0.14	3.12±0.13	77.29±10.02	230.92±11.92	4.13±0.30	1.16±0.10
Trill 3(n=20)	295.84±12.39	2.86±0.24	6.04±0.37	4.90±0.28	1.14±0.25	5.11±0.31	5.34±0.30	94.21±8.63	201.64±9.70	1.00±0.28	0.30±0.13
Trill 4(n=30)	244.86±9.35	2.67±0.22	3.26±0.24	2.48±0.14	0.78±0.14	2.97±0.22	2.87±0.21	53.67±9.06	191.19±10.24	NA	NA
Pulse (n=8)	493.04±159.09	0.99±0.21	NA	NA	NA	NA	NA	NA	NA	NA	NA

3.3.2 Call variants

We subjectively established five Squeak variants, three Trill variants and two chirp variants. However, statistical analyses only confirmed the variants for Squeak and Trill calls, i.e., Chirp variants were not found to be statistically distinguishable. The call variants Squeak 2, Squeak 3, Squeak 4, Trill 1 and Chirp and Pulse calls have not been previously described for Antillean manatees. The variants Squeak 1, Squeak 5, Trill 2, Trill 3, Trill 4 were previously described by Umeed et al. (2018).

3.3.3 Squeak calls

We found that Squeaks were produced by all the study groups (captive females, captive males and males in both reintroduction enclosures). When we considered Squeak variants, we were able to differentiate five variants (Figure 2.2) through the DFA. All variables, with the exception of duration (ms), contributed to the differentiation between the variants (Duration: Wilks' Lambda=0.996; F= 0.170; df1=4; df2= 155; $p < 0.953$; Frequency of maximum energy: Wilks' Lambda= 0.293; F= 93.523; df1=4; df2= 155; $p = 0.017$; Maximum frequency: Wilks' Lambda= 0. 250; F=116.142; df1=4; df2= 155; $p <0.001$; Number of harmonics: Wilks' Lambda= 0.492; F= 40.000; df1=4; df2= 155; $p <0.001$). Function 1 explained 89.6% of the variance, Function 2 explained 7.5% and Function 5 explained 2.9%. We correctly classified 63.1% of Squeak vocalisations using a leave-one-out cross-validated DFA. A PERMANOVA confirmed the accuracy of our Squeak variant categorisation (df = 4, SS= 316.48, MS = 79.12, Pseudo-F = 38.047, p (perm)=0.0001; Unique permutations= 9932). The PERMANOVA showed that we could not differentiate some of the variants, using a pairwise comparison (Table 2.5). However, when considering the enclosure type in a two factor PERMANOVA, Squeak variants could be differentiated (df = 8, SS= 63.79, MS = 7.9737, Pseudo-F = 6.8218, p (perm)=0.0001; Unique permutations= 9912). All Squeaks variants for captive females, captive males and males in reintroduction enclosure 1 could be differentiated in the pairwise comparison (Table 2.6). However, we could not differentiate Squeak 1 from Squeak 2, and Squeak 1 from Squeak 4 for the males in reintroduction enclosure 2 (Table 2.6).

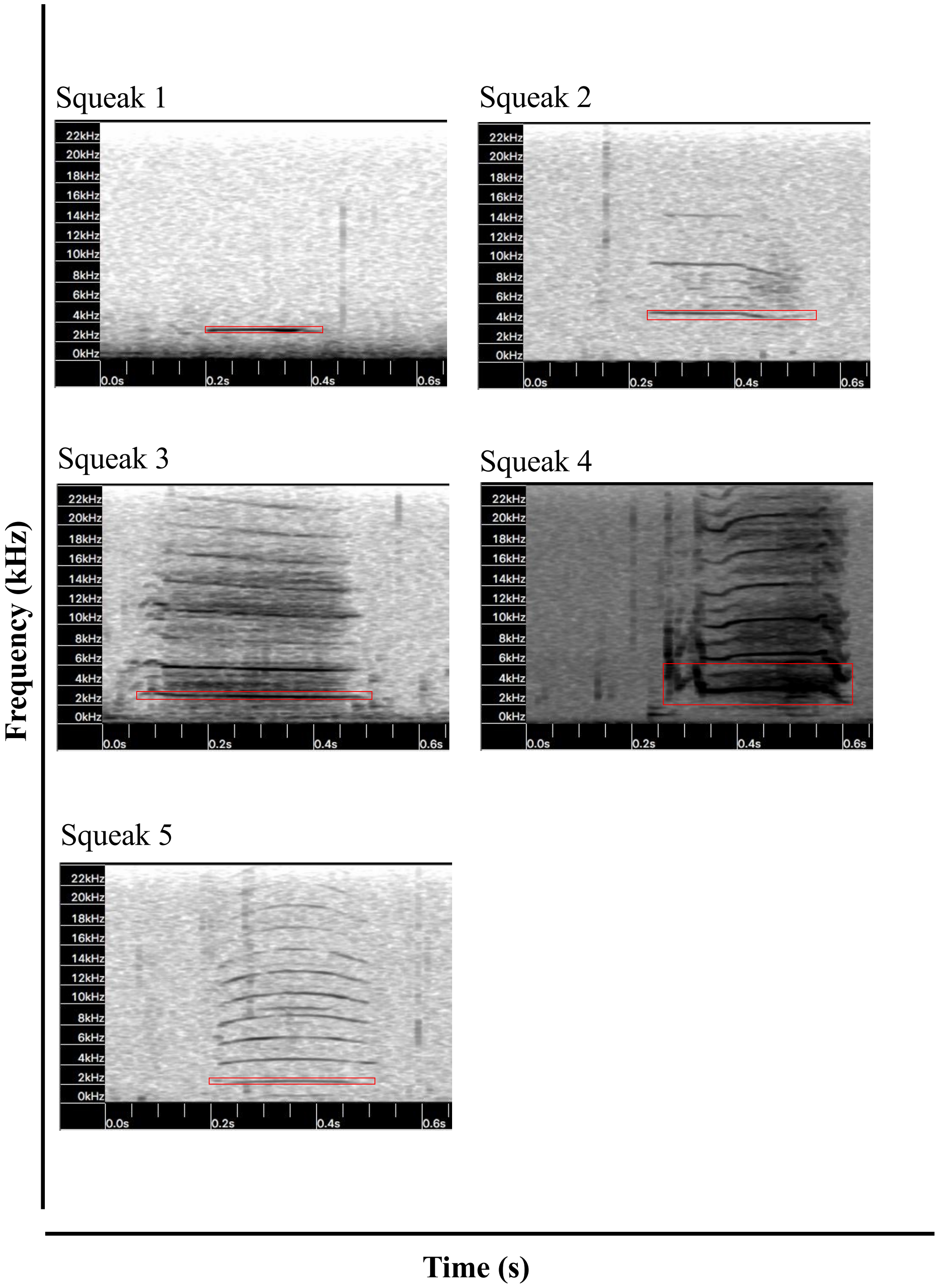


Figure 2. 2. Spectrograms showing the Squeak call category and its variants, Squeaks 1-5. The red rectangles show where the structural variables were measured from for each call. Spectrograms were generated using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33ms, 50% overlap). Squeak 1, Squeak 3 and Squeak 4 were produced by captive

females and males in both reintroduction enclosures. Squeak 2 and Squeak 5 were produced by captive females, captive males and males in both reintroduction enclosures.

Table 2. 5. Results of pair-wise tests indicating the differences between the Squeak variants in relation to each other. The structural variables of the calls used in the comparison were duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and number of harmonics. Underlined p value shows the non-significant result.

Pair wise comparison	t	p(perm)	Unique perms
squeak 1, squeak 2	1.1374	0.2575	9954
squeak 1, squeak 3	9.1366	0.0001	9945
squeak 1, squeak 4	3.1559	0.0003	9954
squeak 1, squeak 5	6.3676	0.0001	9938
squeak 2, squeak 3	7.4299	0.0001	9961
squeak 2, squeak 4	2.7258	0.0014	9953
squeak 2, squeak 5	4.7496	0.0001	9955
squeak 3, squeak 4	10.766	0.0001	9942
squeak 3, squeak 5	3.855	0.0001	9948
squeak 4, squeak 5	7.3689	0.0001	9941

Table 2. 6. Results of pair-wise tests indicating the differences between the Squeak variants in relation to each other. The structural variables of the calls used in the comparison were duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and number of harmonics. Underlined p values show the non-significant results (i.e., p>0.05).

Enclosure / sex	Pair-wise comparison	t	p(perm)	Unique perms
Captive / females	squeak 1, squeak 2	1.9022	0.0108	9471
Captive / females	squeak 1, squeak 3	3.5834	0.0001	9441
Captive / females	squeak 1, squeak 5	2.8351	0.0003	9441
Captive / females	squeak 2, squeak 3	4.695	0.0001	9405
Captive / females	squeak 2, squeak 5	2.2975	0.0043	9461
Captive / females	squeak 3, squeak 5	3.215	0.0001	9435
Captive / males	squeak 1, squeak 2	3.3488	0.0002	9429

Captive / males	squeak 1, squeak 5	7.8573	0.0001	9407
Captive / males	squeak 2, squeak 5	6.9843	0.0001	9376
Reintroduction 1 /males	squeak 1, squeak 3	8.7726	0.0001	9441
Reintroduction 1 /males	squeak 1, squeak 4	2.0859	0.0061	9435
Reintroduction 1 /males	squeak 1, squeak 5	3.712	0.0001	9472
Reintroduction 1 /males	squeak 3, squeak 5	9.1575	0.0002	9420
Reintroduction 1 /males	squeak 3, squeak 5	4.7519	0.0002	9408
Reintroduction 1 /males	squeak 4, squeak 5	4.6335	0.0001	9419
Reintroduction 2 /males	squeak 1, squeak 2	1.673	0.0545	9410
Reintroduction 2 /males	squeak 1, squeak 3	6.2905	0.0001	9378
Reintroduction 2 /males	squeak 1, squeak 4	1.2158	0.2158	9391
Reintroduction 2 /males	squeak 1, squeak 5	5.5944	0.0001	9379
Reintroduction 2 /males	squeak 2, squeak 3	5.3006	0.0004	9436
Reintroduction 2 /males	squeak 2, squeak 4	2.6332	0.0001	9440
Reintroduction 2 /males	squeak 2, squeak 5	4.3875	0.0001	9444
Reintroduction 2 /males	squeak 3, squeak 4	8.0448	0.0001	9394
Reintroduction 2 /males	squeak 3, squeak 5	1.7791	0.0436	9437
Reintroduction 2 /males	squeak 4, squeak 5	8.2108	0.0001	9431

3.3.4 Trill calls

We found that Trills were produced by all the study groups (captive females, captive males and males in both reintroduction enclosures). When considering the Trill variants, we could differentiate three variants (Figure 2.3) using the DFA. Two out of the four variables contributed to the differentiation of the variants (Duration: Wilks' Lambda=0.995; F= 0.173; df1=2; df2= 75; $p < 0.842$; Frequency of maximum energy: Wilks' Lambda= 0.999; F= 0.024; df1=2; df2= 75; $p = 0.976$; Maximum frequency: Wilks' Lambda= 0.877; F=5.244; df1=2; df2= 75; $p <0.007$; Number of harmonics: Wilks' Lambda= 0.810; F= 8.778; df1=2; df2= 75; $p <0.001$). Function 1 explained 65.8% of the variance and Function 2 explained 34.2%. We correctly classified 60.3% of Trill variants using a leave-one-out cross-validated DFA. The PERMANOVA confirmed the accuracy of our Trill variant categorization (df = 2, SS= 51.164, MS = 25.582, Pseudo-F = 13.353, p (perm)=0.0001; Unique permutations= 9941) and showed that it is possible to differentiate Trill variants from one another (Table 2.7).

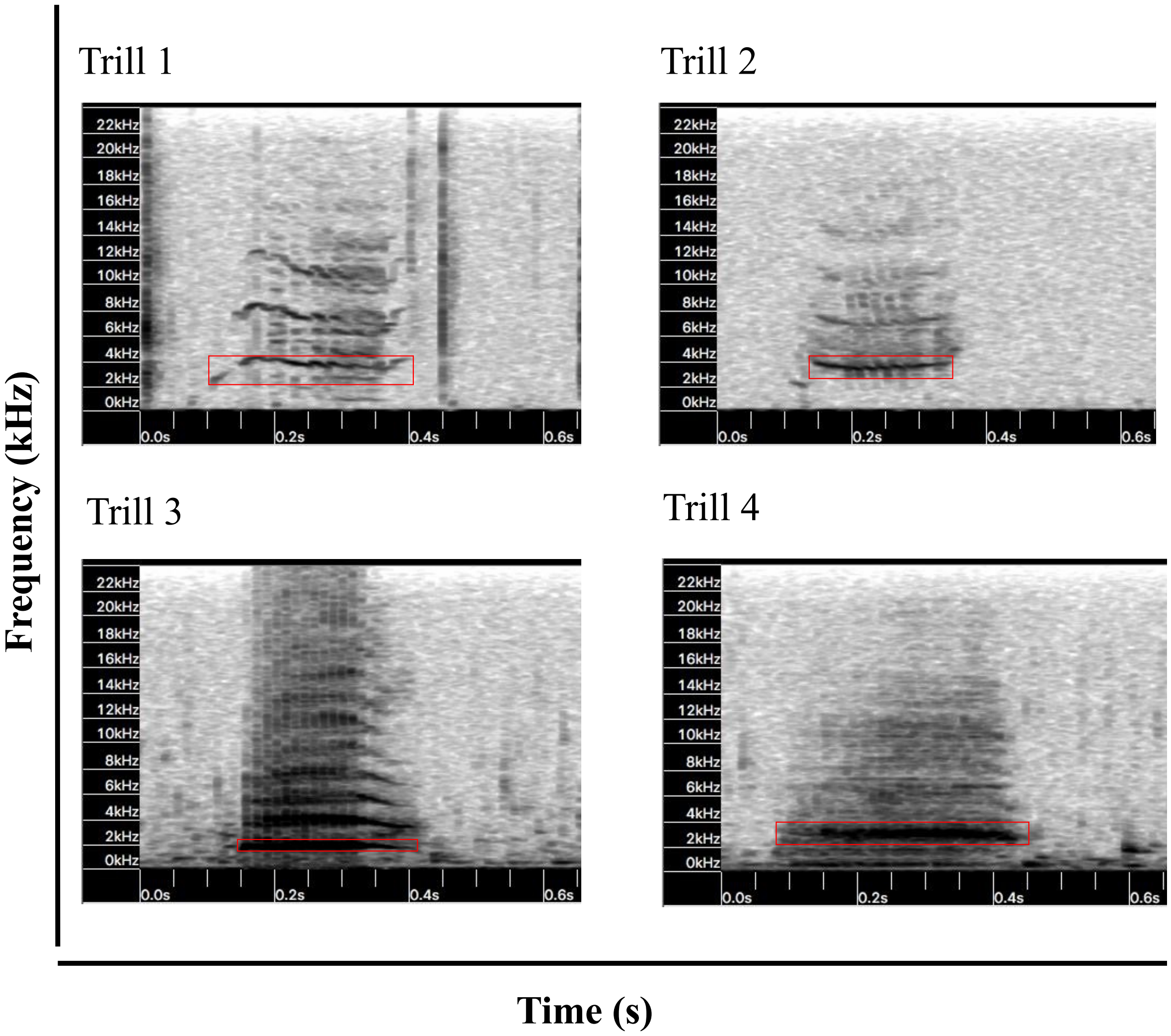


Figure 2. 3. Spectrograms showing Trill calls and its variants, Trills 1-4. Red rectangles show where the structural variables were measured from for each call. Spectrograms were generated using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33ms, 50% overlap). Trill 4 was excluded from the DFA analysis as this variant presents a greater level of chaos and no clearly defined harmonics. Trill 1 was produced by males in both reintroduction enclosures; Trill 2 was produced by all the study groups; Trill 3 was produced by captive males and males in both reintroduction enclosures and Trill 4 was produced by captive females and captive males.

Table 2. 7. Results of pair-wise tests indicating the differences between the Trill variants in relation to each other. The structural variables of the calls used in the comparison were duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and number of harmonics.

Pair wise comparison	**t**	**p(perm)**	**Unique perms**
trill 1, trill 2	2.9976	0.0009	9949
trill 1, trill 3	3.1614	0.0003	9953
trill 2, trill 3	4.2385	0.0001	9959

3.3.5 Chirp calls

We found that Chirps were only produced by males. When we considered the Chirp variants, we could not differentiate the two subjectively categorized variants (Chirp 1 and Chirp 2) (Figure 2.4) using the DFA. None the four variables contributed to the differentiation between the variants (Duration: Wilks' Lambda=.999; F= 0.26; df1=1; df2= 28; $p < 0.872$; Frequency of maximum energy: Wilks' Lambda= 0.910; F= 2.779; df1=1; df2= 28; $p = 0.107$; Maximum frequency: Wilks' Lambda= 0.962; F=1.102; df1=1; df2= 28; $p < 0.303$; Number of harmonics: Wilks' Lambda= 0.971; F= 0.823; df1=1; df2= 28; $p < 0.372$). 53.3% of the calls were categorised correctly by the leave-one-out cross-validated DFA. The PERMANOVA confirmed that our subjective Chirp variant categorization was not accurate (df = 1, SS= 1.5798, MS = 1.5798, Pseudo-F = 1.5616, p (perm)= 0.2055; Unique permutations= 9383).

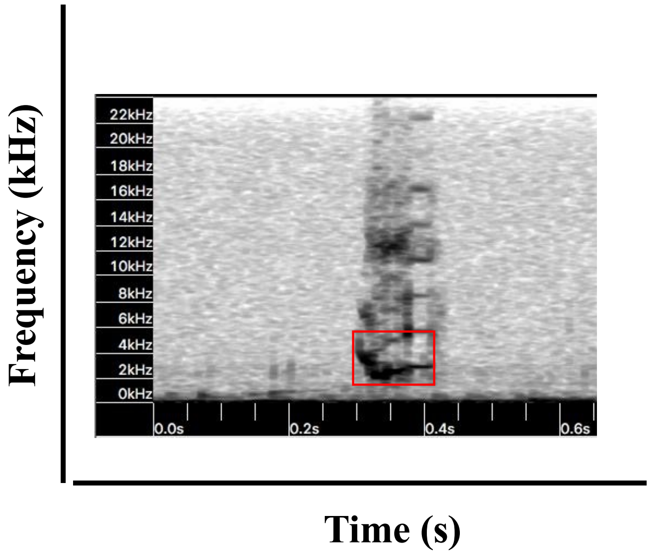


Figure 2. 4. Examples of subjectively categorised Chirp call variants, which were not confirmed using the statistical analyses. Red rectangles show where the structural variables were measured from for each call. Spectrograms were generated using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33ms, 50% overlap). Chirps were produced by the captive males and males in reintroduction enclosure 1.

3.3.6 Pulse calls

We found that Pulse calls were only produced by males. The sample size available for Pulse calls that could be used for the extraction of physical features was small (Table 2.4). Therefore, we provide the initial physical description of the call category as mean ± SE in Table 3-3.1-4 which can be further investigated in the future (Figure 2.5).

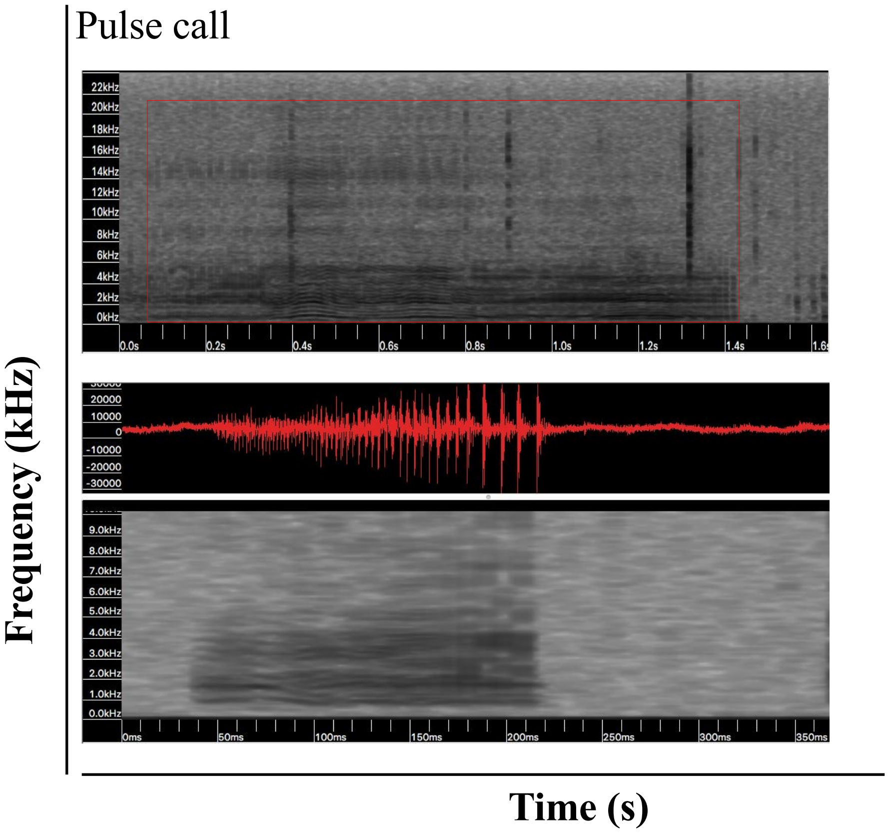


Figure 2. 5. An example of a Pulse call, which were produced by the captive males and the males in reintroduction enclosure 1. Red rectangles show where the structural variables were measured from for each call. Spectrograms were generated using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33ms, 50% overlap). Pulse calls were not included in the DFA analysis as they did not meet the established criteria for the minimum number of calls (n=10) and only two variables were measured for this call type: duration (ms) and frequency of maximum energy (kHz). Here we present the preliminary analysis of this call, which should be further investigated in future studies.

3.3.7 Call and behavioural patterns

All manatee groups produced Squeaks and Trills. Nevertheless, there was a variation in the number of call variants produced according to sex and enclosure type. Captive females produced six variants of two vocalisation types: four Squeak variants and two Trill variants. Captive males produced seven variants of four vocalisation types: three Squeak variants, four Trill variants, Chirps and Pulse calls. The males in reintroduction enclosure 1 produced seven types of variants from three vocalisation categories: four Squeak variants, Chirps and three Trill variants. The males in reintroduction enclosure 2 produced nine types of variants from three vocalisation categories: five Squeak variants, four Trill variants and Pulse calls.

The call rates (i.e., number of calls per number of animals in the enclosure per observational session) differed between captive females, captive males, males in reintroduction enclosure 1 and reintroduction enclosure 2 (df = 3, SS= 2795.8, MS = 931.93, Pseudo-F = 38.721, p (perm)=0.0001; Unique permutations= 9955). Pair-wise tests indicated that the call rates of each group varied in relation to each other (Table 2.8). The call rates for captive females, captive males, males in reintroduction enclosure 1 and males in reintroduction enclosure 2 were 3.87, 1.99, 12.19, and 6.83 calls per animal per observational session, respectively.

Table 2. 8. Results of pair-wise tests indicating that the call rates of each group varied significantly in relation to each other, $p<0.05$.

Pair wise comparison	**t**	**p(perm)**	**Unique perms**
Captive females, Captive males	2.8641	0.0034	1149
Captive females, Reintroduction 1	8.5683	0.0001	1068
Captive females, Reintroduction 2	3.2107	0.0015	952
Captive males, Reintroduction 1	10.581	0.0001	718
Captive males, Reintroduction 2	5.1524	0.0001	575
Reintroduction 1, Reintroduction 2	4.2161	0.0001	394

We found significant differences in the call patterns of the different study groups when considering the proportion of each call category produced (Squeaks, Trills, Chirps, Pulses) (Chi square test: 1027.35; gl: 9; $p< 0.001$; Figure 2.6). The behavioural patterns also differed between the groups (Chi square test: 456.79; gl: 24; $p< 0.001$; Figure 2.7).

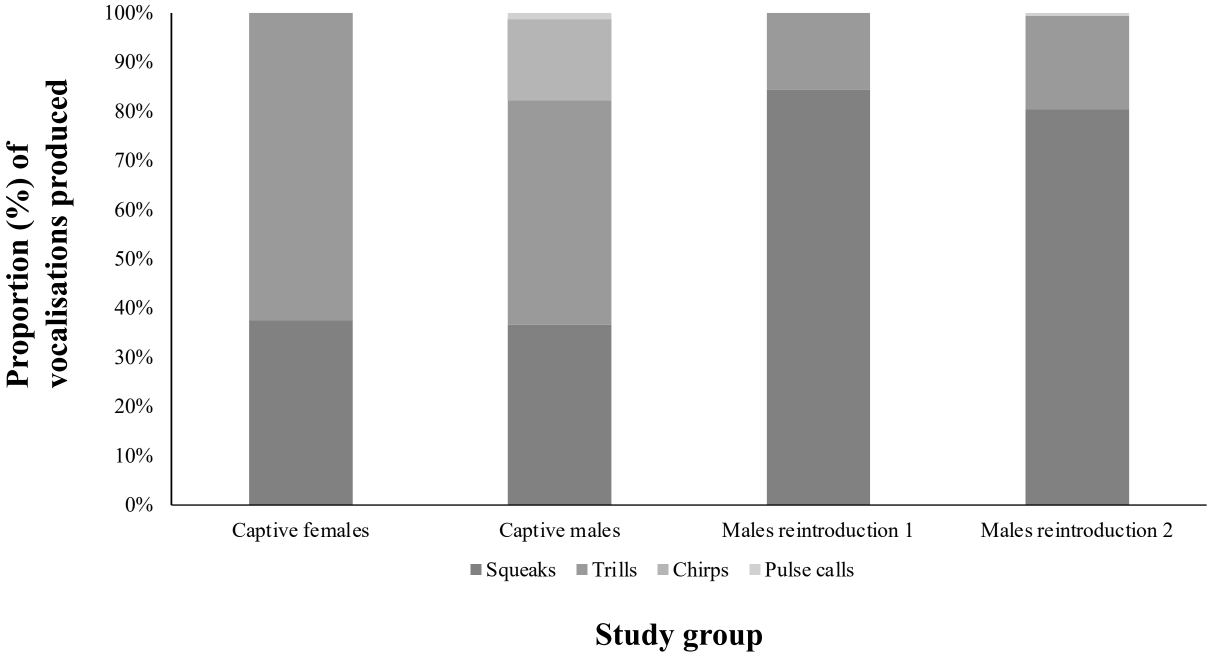


Figure 2. 6. Proportion (%) of vocalisations produced by each study group, captive females, captive males, males in reintroduction enclosure 1 and males in reintroduction enclosure 2. The proportion of vocalisations produced by each group were significantly different for each call type recorded.

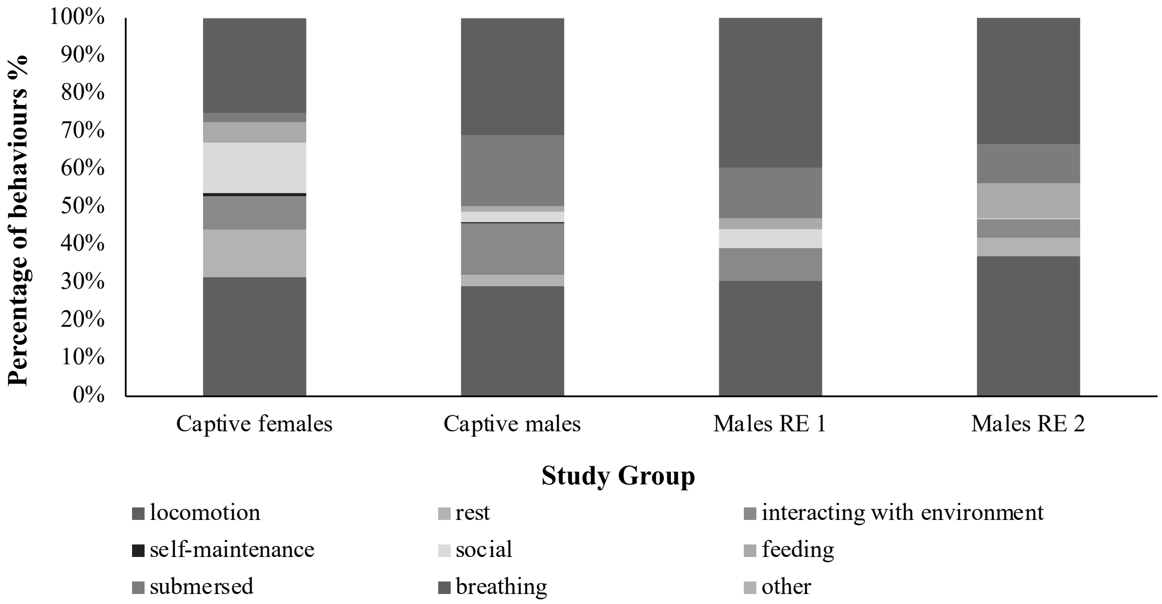


Figure 2. 7. The observed focal behaviours during the recordings for each study group (captive females, captive males, males in reintroduction enclosure 1 (Males RE1) and males in reintroduction enclosure 2 (Males RE 2). The ethogram used here followed behavioural definitions by Umeed et al. (2018) (locomotion, rest, social, feeding, submersed and other) and Lucchini et al. (2021) (interaction with environment, self-maintenance). The proportion of behaviours demonstrated for each study group were significantly different.

3.3.8 Individual differences in Squeak structure: vocal signature

We found that each animal produced a Squeak vocalisation that consistently had the same basic structure (Figure 2.8), but that often varied in terms of the number of harmonics and duration (Table 2.9).

We found that all variables, except call duration (ms) contributed to the differentiation between the individual manatees (Duration: Wilks' Lambda=0.869; F= 1.812; df1=2; df2= 36; p = 0.162; Frequency of maximum energy: Wilks' Lambda= 0.467; F= 13.694; df1=2; df2= 36; p = <0.007; Maximum frequency: Wilks' Lambda= 0.220; F=42.460; df1=2; df2= 36; p <0.001; Number of harmonics: Wilks' Lambda= 0.671; F= 5.894; df1=2; df2= 36; p <0.002). Function 1 explained 87.3% of the variance, Function 2 explained 7.4%, and Function 3 5.2%. We correctly classified 80.0% of individual manatee vocalisations using a leave-one-out cross-validated DFA (Figure 2.9). The PERMANOVA indicated a highly significant difference among the animal vocalisations (d.f. = 3, SS = 93.41, MS = 31.14, Pseudo-F = 17.91, p=0.0001, Unique permutations = 9944) and all animals presented significant differences in relation to each other (Table 2.10). The PERMDISP analysis indicated no significant difference of multivariate dispersion among the animals (F = 1.03, d.f.1=3, d.f.2= 36, p>0.05).

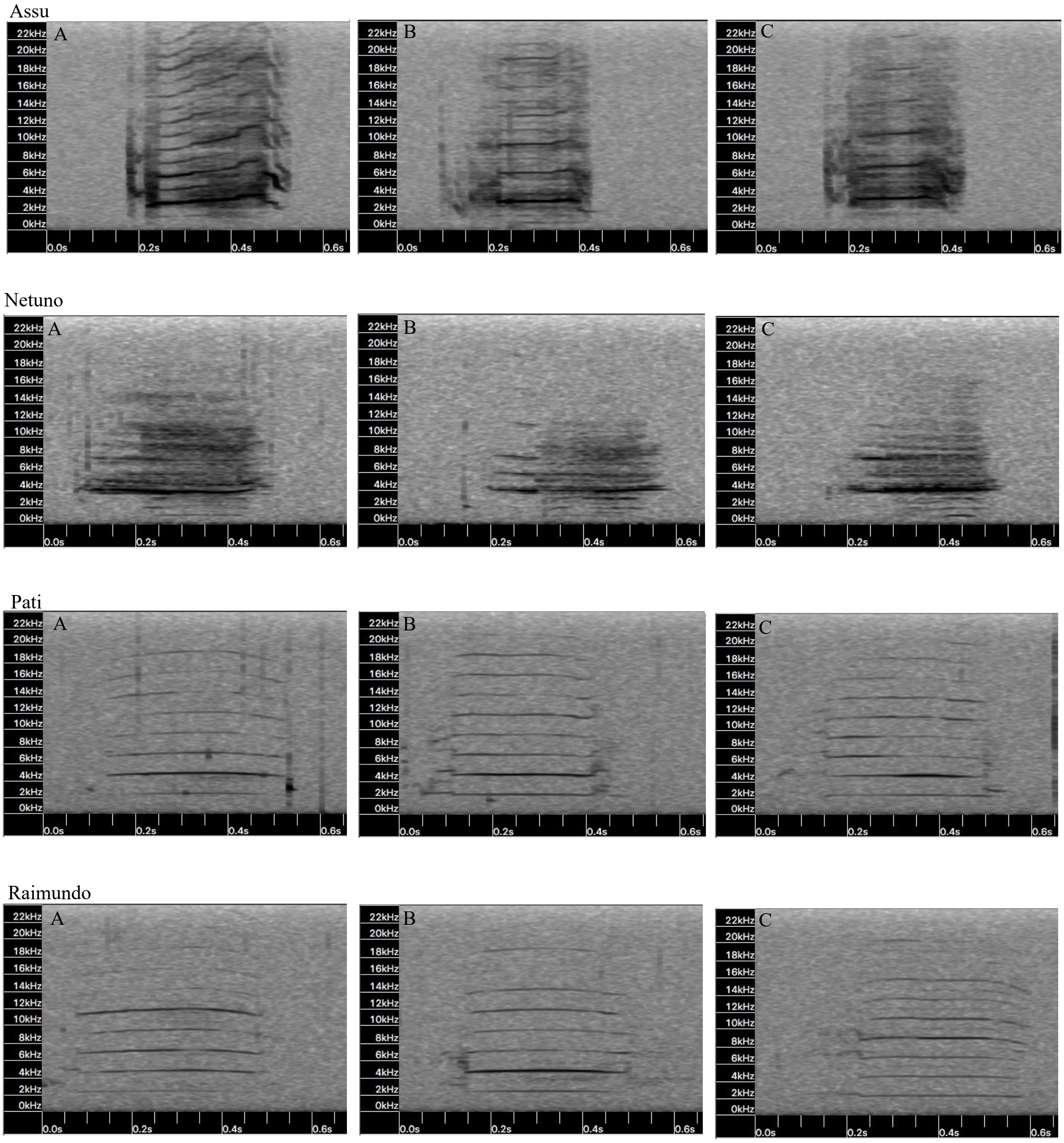


Figure 2. 8. Inter and intraindividual differences (a, b, c) in the squeak calls produced by Assu, Netuno, Raimundo and Paty. Spectrograms were also generated using Kaleidoscope Pro5 to visually illustrate the individuality observed for the individual-specific vocalisations.

Table 2. 9. Inter and intraindividual differences (a, b, c) in the squeak calls produced by Assu, Netuno, Raimundo and Paty. Spectrograms were also generated using Kaleidoscope Pro5 to visually illustrate the individuality observed for the individual-specific vocalisations produced by each of the four study animals.

Animal (n. vocalisations)	**Syllable Duration (ms)**	**Frequency of maximum energy (kHz)**	**Maximum Frequency (kHz)**	**Minimum Frequency (kHz)**	**Bandwidth (kHz)**	**Start frequency (kHz)**	**End frequency (kHz)**	**Duration from start to maximum frequency (ms)**	**Duration from maximum frequency to end (ms)**	**Number of harmonics**	**Frequency interval between harmonics (kHz)**
Assu (n=10)	320.06±11.08	3.59±0.11	5.09±0.17	2.90±0.20	2.19±0.23	4.10±0.23	2.98±0.23	41.93±17.85	278.14±16.26	7.80±1.0	0.08±0.34
Netuno (n=10)	396.05±9.52	1.39±0.31	1.64±0.30	1.43±0.33	0.21±0.04	1.48±0.32	1.45±0.33	55.52±11.36	340.52±14.02	11±0.54	0.45±0.06
Raimundo (n=10)	470.92±18.81	2.25±0.08	3.38±0.16	2.40±0.04	0.98±0.17	3.31±0.19	2.55±0.12	27.52±14.02	443.40±25.58	8.3±0.45	1.03±0.18
Paty (n=10)	391.37±22.64	2.36±0.01	2.77±0.06	2.40±0.04	0.37±0.07	2.77±0.06	2.52±0.05	0	391.37±22.64	7.7±0.37	1.46±0.13

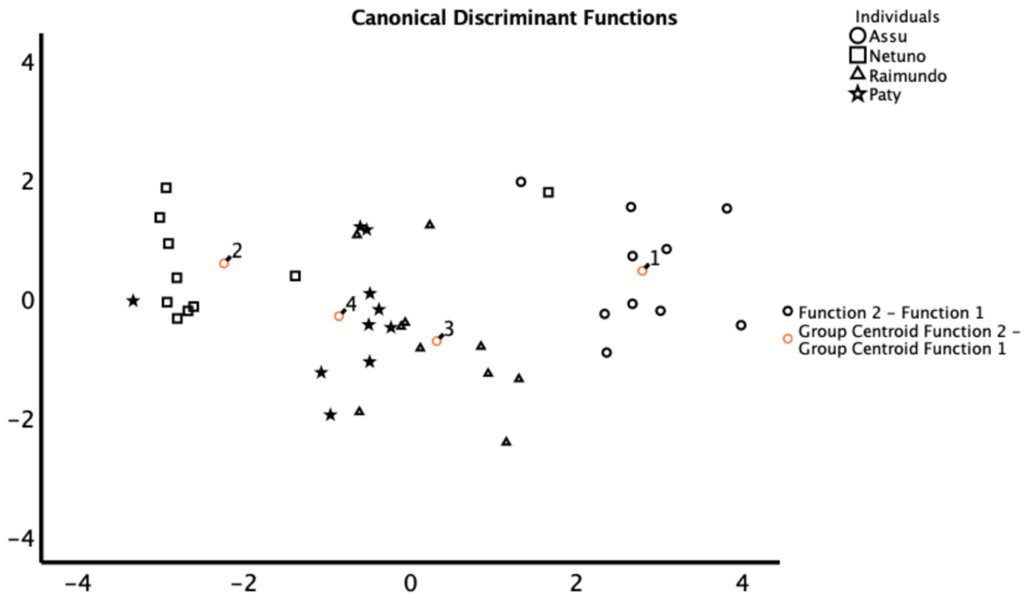


Figure 2. 9. Distribution of the discriminant scores for the individual calls produced by the four study manatees in the reintroduction enclosures along two canonical discriminant functions established to discriminate among the vocalisations. The scatterplot demonstrates the classification of the vocalisations produced by individuals in the reintroduction enclosures.

Table 2. 10. Results of pair-wise tests indicating significant differences of the individual manatee vocalisations in relation to each other. The structural variables of the calls used in the comparison were duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and number of harmonics.

Group	**t**	**P (perm)**	**Unique permutations**
Assu, Netuno	5.64	0.0001	9419
Assu, Raimundo	4.67	0.0001	9416
Assu, Paty	3.91	0.0001	9448
Netuno, Raimundo	3.71	0.0001	9449
Netuno, Paty	3.47	0.0001	9421
Raimundo, Paty	2.43	0.0099	9395

3.4 Discussion

Here we demonstrate complexity in different aspects of Antillean manatee vocal behaviour. We described four call categories and their variants produced by Antillean manatees living in captive and reintroduction enclosures and experiencing different artificial social contexts established by enclosure type. Two of the call categories are newly described for Antillean manatees (i.e., Chirps and Pulse calls), which were produced exclusively by the captive and reintroduction males in our study. Chirp calls have been previously described by Brady et al. (2020) for Florida manatees, *Trichechus manatus latirostris,* however, no information on sex-specificity was provided in the study. Two of the call categories (Squeaks and Trills) presented distinguishable variants, some of which were also newly described for Antillean manatees. However, not all call variants were produced by all social groups in our study.

Overall, the study manatees presented different call repertoires, call structures and call production according to their social context. The ability to adjust vocalisations depending on environments and social contexts can be useful for optimising communication (Hanna et al., 2014, Gill et al., 2015). This ability may be particularly useful for manatees since they often move between areas of mangroves, open sea and estuaries and therefore, experience physical changes in their environments (Favero et al., 2020; Medeiros et al., 2021; Santos et al., 2022) and can have varied group configurations (Ramirez-Jimenez et al., 2017). The ability to vary vocal production and structure may be especially useful when trying to avoid the degradation of physical call attributes during propagation due to variations in water temperature, pH (Ilyina et al., 2009) and shallow depths (Ramos et al., 2020) or masking of vocalisations by ambient noise (Miksis-Olds & Tyack, 2009). Many species are known to alter their vocalisations (rate and physical structure) to compensate for cue-masking effects in noise polluted environments (Berger-Tal et al., 2019).

On the other hand, some level of vocal stability could be potentially beneficial for communicating in different social contexts (Rekdahl et al., 2013). This may be the case for Squeak calls, which were produced by all the study groups and whose variants were only distinguishable when considering enclosure type. Squeak variants could be differentiated in all enclosures, except in reintroduction enclosure 2, which housed two young males, where three of the Squeak variants were not distinguishable. However, some factors that were not accounted for in our study, such as pH, salinity, turbidity, tide level, as well as enclosure depth and wall material, could have affected the physical structure of the vocalisations recorded. Chavarría et al. (2015), found that the sound transmission of Antillean manatees is influenced by estuarine processes, as well as estuarine sedimentary cumulative effects and concluded that frequency transmission is mainly dependent on river depth and bottom characteristics. Additionally, studies in marine environments have demonstrated that sound absorption decreases with increasing temperatures, as well as with decreasing pH, facilitating underwater sound propagation (Ilyina et al., 2009). As such, natural, as well as anthropogenic noises, will travel further, resulting in an increase in underwater noise levels (Klopper & Simmonds, 2014; Gazioglu et al., 2015). Therefore, it is important to consider the effects of abiotic water features on manatee vocalisation structure in future studies.

The repertoire of call variants also varied between the animal groups, with the young males in reintroduction enclosure 2 having the most varied repertoire, producing three types of calls and nine call variants. This result confirms the idea that age may play a role in call production in Antillean manatees (Sousa-Lima et al., 2008; Umeed et al., 2018). The captive females had the least varied repertoire producing only two types of vocalisations, thus supporting our first prediction that females would produce more stereotypical (i.e., simpler) repertoires. Furthermore, since several Squeak and Trill variants and two call categories (i.e., Chirp and Pulses) were found to be exclusively produced by males, we reinforce the findings of previous studies that have demonstrated that sex influences vocalisation production in Antillean manatees (Umeed et al., 2018). Green (1981) notes that specific morphologies in the vocal repertoire of one sex may be absent in the other. These differences may be due to anatomical dissimilarities, which result in the production of a specific acoustic shape by only one of the sexes (Green 1981). Green (1981) further notes that such differences would be expected to be seen in animals that have reached maturity. However, if these differences are associated with control of the phonatory apparatus rather than anatomical dissimilarities, they would be observed in early puberty or even before (Green 1981). Such anatomical differences have not yet been observed for West Indian manatees, *Trichechus manatus,* however research on the topic is limited (Murie, 1872; Landrau-Giovanetti et al., 2014). Currently, research has shown that the larynx of manatees is structurally similar to that of terrestrial mammals and that vocal folds appear to be the primary mechanism for sound production in manatees, however sexual dimorphism in manatee vocal mechanisms has not yet been addressed (Landrau-Giovanetti et al., 2014)

The overall call rate varied between the study groups. We found that older males in reintroduction enclosure 1 had the highest call rate followed by the younger males in reintroduction enclosure 2, the captive females and the captive males. This was contrary to our first and second predictions, where we expected females to produce the greatest number of vocalisations, since the captive females comprised the study group with the greatest number of individuals. Thus, Antillean manatees may not conform to the entirety of the social complexity hypothesis for communication (see Freeberg et al., 2012 for a review of the different predictions of this hypothesis). It is possible that it was not energetically beneficial nor necessary for females to maintain vocal contact with one another as they were constantly in close proximity to one another in their captive enclosure. On the other hand, males in the reintroduction enclosures had a greater amount of space and a lower number of individuals in the enclosure, potentially creating a more complex social scenario i.e., the males were able to separate and come together more freely, likely requiring greater vocal production in order to maintain contact. Studies in captivity have also demonstrated that manatees exhibit more social behaviour and vocalise more during the night compared to during the day (Hénaut et al., 2010; Costa et al., 2018). Therefore, the fact that this study was conducted during the day may also help to explain the lower call rates recorded for the captive females.

We found that the proportion of use of the different call categories varied between the four groups, which reflects the different behavioural patterns observed for each group. Squeaks were produced significantly more by the males in the readaptation enclosures, compared to the animals in the captive enclosures and Trills were produced significantly more by captive animals. Brady et al. (2021), found that animals under greater levels of stress almost exclusively produced Squeak vocalisations, whereas animals produced "Squeals", described here as Trills, during cavorting behaviours. This may explain the differences in vocal call type production observed here. Sex, social context and enclosure limitation may explain this result. Green (1981) organised sex-specific differences in animal acoustic signals into three main categories: 1) vocalisations that are produced by one sex and not the other; 2) vocalisations that are produced by both sexes, however, differ in their usage; and 3) vocalisations produced by both sexes yet, have different structures that result from sexual dimorphism in the structure and size of sound-producing organs (Sayigh et al., 1995). The first and the third differences described by Green (1981), were demonstrated by Umeed et al. (2018) for Antillean manatees, in terms of vocal production since, as of yet, no anatomical studies have been performed on sexual dimorphism in sound-producing organs. However, here we provide indirect evidence of Green's second category of differences for Antillean manatees the first time. Our results suggest that the vocalisations that are produced by both sexes (i.e., Squeaks and Trills) differ in their usage – i.e., animals of different sexes with different behavioural patterns present different call patterns. Nevertheless, studies focusing on recording specific behaviours and vocalisations from specific focal individuals (rather than a focal group) would be the ideal scenario to test this theory of sex-related differences, suggested by Green (1981). Such ideal observational conditions, however, may not be easily obtained (with Antillean manatees housed in captive, natural reintroduction or free-living scenarios) in Brazil without causing potential isolation-related stress in the animals.

3.4.1 Individual differences in vocalisation structure

We found that the four individuals, located in the reintroduction enclosures in the year 2020, produced calls with unique physical structures, thus supporting our final prediction that Antillean manatee vocalisations differ structurally between individuals. We found that the vocalisations produced by Netuno had the lowest average maximum frequencies and the lowest frequency range. Netuno was the largest and oldest of the individually recorded animals, which may explain the low frequencies observed in the signature calls of this manatee. Studies have demonstrated that body size and call frequency are negatively correlated (Mathews et al.,1999; May-Collado et al., 2007; Sousa-Lima et al., 2008; Dunn et al., 2017). However, if we consider a simple correlation between the BMI and the average highest frequency of Squeak calls from the reintroduction animals recorded individually, we see no correlation (Spearman's rho -0.600, n= 4, p= 0.40). Additionally, Paty, the smallest individual, produced calls with unexpectedly low average high frequencies. The calls produced by Raimundo and Paty have highly similar structures which suggests that the signature vocalisations of manatees may be learned as calves or may be defined during the juvenile development stage and likely simulate the acoustic signals of related and/or unrelated individuals (Green, 1981; Sousa-Lima et al., 2008). This phenomenon has been recorded for bottlenose dolphins, *Tursiops truncatus* (Fripp et al., 2005), as well as for Blainville's beaked whales, *Mesoplodon densirostris* (Dunn et al., 2017) and killer whales, *Orciuns orca* (Nousek et al., 2006). Paty was raised in isolation at the ICMBIO/CMA for three years and was then moved to the female oceanarium. She was then translocated to reintroduction enclosure 2 when she was four years old and was placed together with Raimundo. Thus, Paty and Raimundo spent a period of 1 year together at the time of our recordings. However, further studies are required to investigate at what age signature vocalisations in manatees become defined, if at all.

Individuality in vocalisations has been identified for several other species of marine mammals, such as bottlenose dolphins, *Tursiops truncatus* (Caldwell & Caldwell, 1979); free-ranging common dolphins, *Delphinus delphis* (Fearey et al., 2019); Indo-Pacific bottlenose dolphins, *Tursiops aduncus* (Gridley et al., 2014); captive beluga whales, *Delphinapterus leucas* (Morisaka et al., 2013) and narwhals, *Monodon monoceros* (Shapiro, 2006). The widespread occurrence of signature vocalisations highlights their importance for individual survival. Across mammalian species, the most common infant isolation call is a long, tonal call which effectively recruits help from mothers (Zeifman, 2001; Newman, 2004; Newman, 2007; Lingle et al., 2012). This apparent requirement of some level of vocal stability may introduce the idea of signature vocalisations for mammalian species, i.e., vocalisations that remain the same independent of social and physical contexts (Rekdahl et al., 2013). This could suggest the use of Squeaks as potential signature vocalisations in manatees, as they generally have longer durations and are more tonal, compared to the other types of vocalisations described here. Our findings introduce the possibility of creating an individual vocalisation database for manatees in north-eastern Brazil, where reintroduced and wild manatee vocalisations could be recorded and archived for monitoring purposes. This would facilitate governmental and independent agencies in identifying and monitoring individual animals and may be a potentially important tool for the conservation of north-eastern manatee populations.

4 Article 2 - Acoustic interactions between free-living mother-calf Antillean manatees, *Trichechus manatus manatus*

4.1 Introduction

Vocal communication between mothers and calves occurs in several marine mammal species (Tables 3.1, 3.2), including reports for manatees (Hartman, 1979; Reynolds III, 1981; Mann et al., 2006; O'Shea and Poché, 2006; Gerstein et al., 2008). Many studies have attempted to determine the information contained in the acoustic signals exchanged between mothers and calves, highlighting the importance of vocal communication in infant survival. For instance, in Florida manatee (*Trichechus manatus latirsotris*), calves vocalise more than mothers (Hartman, 1979). Calls from Florida manatee calves have a hill-shaped structure and the mothers' calls have a flatter structure (O'Shea and Poché, 2006). Furthermore, calls are considered important in mother-calf individual recognition, with calls preceding movement until mothers and calves are reunited (O'Shea and Poché, 2006). Several marine mammal species can produce signature vocalisations (e.g., Tables 3.1, 3.2), which consist of calls with unique spectral and temporal features that can encode individual or group identity (Shapiro 2006). Signature vocalisations may facilitate mother-offspring contact, individual recognition and reunion (King et al., 2016).

Antillean manatees, *Trichechus manatus manatus*, occur in North-eastern Brazil and are currently under threat of extinction (MMA 2014). Captive studies have shown that manatees produce vocalisations with fundamental frequencies ranging from 0.64kHz-8.1kHz, which can contain individual sender information such as age and gender (Sousa-Lima et al., 2008; Chavarría et al., 2015; Umeed et al. 2018). However, the vocalisations of wild Antillean manatees in Brazil, especially vocal interactions between mothers and calves, are poorly understood.

Table 3. 1. Acoustic interactions between mothers and calves and the production of signature/contact calls. We performed a systematic review of publications available on the platforms Google Scholar and Pubmed using the keywords: “Mother calf acoustic interactions “Mother calf contact calls”, “Mother calf vocal interactions”, “Mother and calf calls dolphins”, “Mother and calf calls whales”, “Mother and calf calls porpoises”, “Mother calf vocal interactions sperm whales”, “Mother calf acoustic behaviour marine mammals”. All of the species were found to produce signature vocalisations or contact calls, with the exception of *Globicephala melas*.

Species	**Production of signature/contact call**	**Type of signature/contact call**	**Source**
Balaena edeni	Yes	'onal calls and broadband pulses	Edds et al., 1993; De Figueiredo and Simão, 2014.
Balaena mysticetus	Yes	Pulsed and upsweep calls	Ljunblad et al.,1 980 Wursig et al., 1985
Delphinapterus leucas	Yes	Pulsed sounds, frequency-modulated sounds and repetitive click trains	Van Parijs et al., 2002; Morisaka et al., 2013
Eubalaena australis	Yes	Frequency modulated upsweep vocalisation	Clark, 1983
Eubalaena glacialis	Yes	Upcalls	McCordic et al., 2016; Parks et al., 2019
Globicephala melas	No	Low amplitude calls	Zwamborn and Whitehead, 2017
Megaptera novaengliae	Yes	Social calls (bass, boom, wop and trill)	Dunlop et al., 2008; Saloma, 2018; Indeck et al., 2020
Neophoca cinerea	Yes	Harmonic calls	Charrier & Harcourt, 2006
Odobenus rosmarus rosmarus	Yes	Distress barks, soft barks	Charrier et al., 2010
Orcinus orca	Yes	Clicks, whistles, pulsed, signals	Weiß et al., 2005
Phocoena phocoena	Yes	Clicks	Clausen et al., 2012
Tursiops aduncus	Yes	Whistles	Gridley et al., 2014
Tursiops truncatus	Yes	Whistle	Caldwell et al., 1990; Sayigh 1990; Sayigh et al., 1995; Tyack, 1997; Fripp et al., 2005)

Table 3. 2. Signature vocalisations and/or individuality previously observed for marine mammal species. We reviewed the publications available on the platforms Google

Scholar and Pubmed was performed using the keywords: “Signature vocalisations whales”, “Signature vocalisations whales”, “Signature vocalisations dolphins”, “Signature vocalisations marine mammals”, “Signature calls marine mammals” and “Vocal individuality marine mammals”.

Species	**Type of signature vocalisation**	**Variables analysed**	**Source**
Arctocephalus tropicalus	Tonal calls	Frequency and frequency of peak amplitude	Charrier et al., 2003
Delphinus delphis	Whistles	Highly similar frequency modulation patterns	Fearey et al., 2019.
Delphinapterus leucas	Pulsed sound	Pulse repetition rate and pattern	Morisaka et al., 2013; Mishima et al., 2015
Eubalaena glacialis	Upcalls	Frequency, spectral entropy, duration	McCordic et al., 2016
Leptonoychotes weddellii	“Primary” calls	Duration,	Collins et al., 2006
Megaptera novaengliae	Social calls (bass, boom, wop, trill)	Call type and amplitude	Saloma, 2018
Monachus monachus	Barks and screams	Duration and minimum bandwidth	Charrier et al., 2017
Monodon monoceros	Combined tonal/pulsed signals and whistles	Average IPI, duration, number of pulses, pulse repetition rate, fundamental frequency contour	Shapiro, 2006
Neophoca cinerea	Harmonic calls	Fundamental frequency, energy spectrum, frequency and amplitude modulation.	Charrier & Harcourt, 2006
Orcinus orca	Stereotyped calls	Frequency contours	Nousek et al., 2006
Physeter macrocephalus	Codas	Inter-pulse interval, inter-click interval	Oliveira et al., 2016
Sotalia guianensis	Whistles	Frequency contour	Figueiredo & Simão, 2009
Tursiops truncatus	Whistles	Loop repetition, frequency modulation, frequency contour	Caldwell et al., 1990; Sayigh 1990; Sayigh et al., 1995; Tyack, 1997; Fripp et al., 2005)
Tursiops aduncus	Whistles	Contour categorisation and bout analysis	Gridley et al., 2014

In this study, we aimed to detail rare recordings of the vocalisations produced by three free-living Antillean manatee mother-calf pairs and one trio, inhabiting estuarine areas in North-eastern Brazil. Here we describe call rates and call types produced by the study mothers and calves. We also investigated whether we could differentiate the mother-calf pairs based on the structure of Squeak vocalizations – a call type that varies in structure and production depending on the behaviour, individual, sex and/or habitat in Antillean and Florida manatees (O'Shea and Poché, 2006; Sousa-Lima et al., 2008; Chavarría et al., 2015; Umeed et al., 2018; Brady et al., 2021). Thus, we expected that the vocalisations produced by different mother-calf pairs would have different physical structures.

4.2 Materials and Methods

4.2.1 Study area

We conducted fieldwork in two areas in North-eastern Brazil: the Mamanguape River, Paraíba state (6.78°S, 34.93°W) and the Riacho Tabatinga, Alagoas state (9.22°S, 35.33°W) (Figure 3.1).

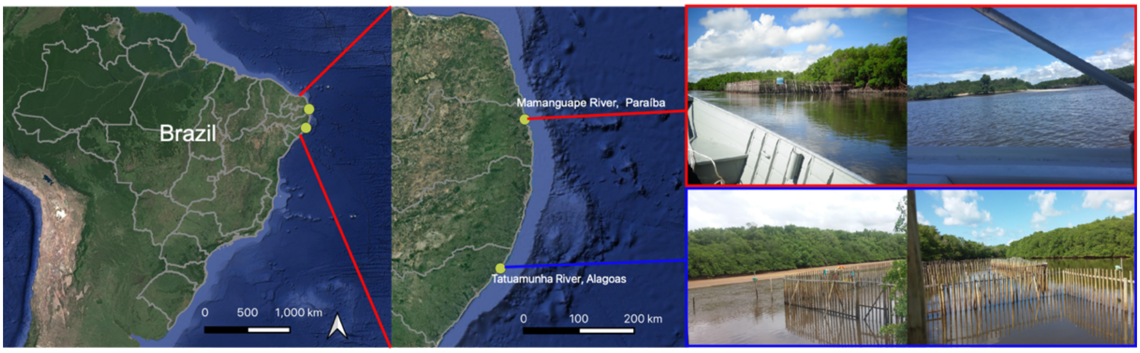


Figure 3. 1. Figure illustrating the sites where the mothers and calves were recorded in the Mamanguape River, Paraíba (6.78□S, 34.93□W) and the Tatuamunha River, Alagoas (9.22□S, 35.33□'W), North-eastern Brazil. Sources: maps obtained using QGis 3.22.0 Białowieża Google Satellite, photos taken by RU.

4.2.2 Study animals

We recorded the vocalisations from three mother-calf pairs and one trio (Table 3.3) without interruptions using a Cetacean Research SQ26-H1 hydrophone connected to a Zoom H1 recorder (linear frequency response: 20Hz-45kHz, +3/12dBs; sampling frequency 48 kHz 16 bits). All mothers and calves were free-ranging and had no telemetry equipment to help locate the animals; thus, they were recorded when eventually encountered during active searches by boats during our field expeditions.

4.2.3 Vocalisation analysis

The vocalisations were categorised by one single researcher, RU, based on Fitch et al. (2002), Mann et al. (2006), Buder et al. (2008), Sousa-Lima et al. (2008), Umeed et al. (2018), Brady et al. (2020) and Umeed et al. (2023). The calls were first classified visually using spectrograms and waveforms created by Kaleidoscope Pro5, Wildlife Acoustics. RU classified all the calls. Thus, vocalisations were classified as Squeaks, Trills, Squeak-Trills Chirps, Pulse calls or Rubbing (Figure 3.2.). Here we grouped Umeed et al.'s (2018) screeches, creaks and trills into a single group of calls with a certain degree of chaos (Trills) and/or biphonations (Mann et al., 2006; Buder et al., 2008). Chirps followed Brady et al.'s (2021) classification. Pulse calls were defined following Umeed et al. (2023). Squeaks were defined as harmonic, modal vocalisations (Sousa-Lima et al., 2008; Umeed et al., 2018; Brady et al., 2020; Umeed et al. 2023). The physical structures of vocalisations were analysed using Kaleidoscope Pro5, Wildlife Acoustics software. Measurements were only taken from the fundamental harmonic of vocalisations where the full structure was visible and clear. RU measured eleven physical structural variables (3.4) from the fundamental frequency of each vocalisation produced by each mother-calf pair. It was not possible to record the mothers and calves separately in order to determine which vocalisations were produced by which animals.

We calculated the call rates (number of vocalisations/minute) for each of the mother-calf pairs (i.e., the Mamanguape river pair and the two Riacho Tabatinga pairs Aira and Bacuri, and Aira and the new calf) and the Riacho Tabatinga trio (i.e., Aira, older offspring Bacuri and the new calf together). To verify whether vocalizations could be separated into distinct call types, we used a permutational analysis of variance (PERMANOVA) with Euclidean distance, using Primer 6 software (Anderson et al., 2008). We used all call parameters available to conduct the PERMANOVA. We also generated spectrograms to demonstrate individuality in the mother-calf vocalisations. Additionally, we performed a Discriminant Function Analysis (DFA) using SPSS v27, IBM, to investigate whether it was possible to discriminate the mother-calf unities based on the physical structure of the Squeak vocalisations that they produced. All the Squeak vocalisations recorded from each study site were included in the DFA, and we considered the structural variables duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz), in the analysis. We used about a third of the total number of call variables available in our DFA to reduce the likelihood of misleading the classification of the calls (Lachenbruch and Goldstein 1979; Kazial et al., 2001). We also performed a Permutational Multivariate ANOVA (PERMANOVA) with Euclidean distance, using Primer 6 software (Anderson et al., 2008), to test the statistical validity of calls assigned to each mother-calf unity. Furthermore, permutational t-tests were used for *post hoc* pairwise comparisons to verify whether each mother-calf unit differed from each other. For the PERMANOVA, we considered all Squeak call variables available.

Table 3. 3. Mother-calf pairs and mother-calf trio recorded during eventual encounters in North-eastern Brazil during fieldwork expeditions.

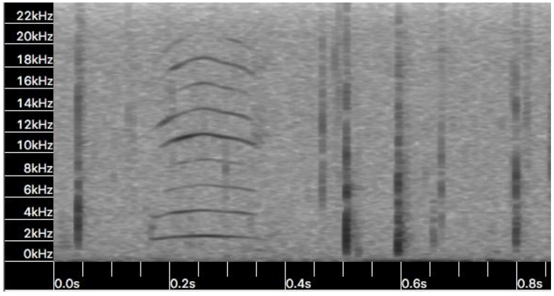
Mother calf unit	**Location**	**Recording period and conditions**
Mother-calf pair 1	Gamboa de Caracabu and Mamanguape River, Paraíba	We recorded the mother-calf pair, from 0900h to 1120h on 9th July 2019, starting in the amboa de Caracabu and accompanying the mother-calf pair down the Mamanguape River during high tide (Figure 3.1). A total of 2 hours and 20 minutes of uninterrupted acoustic signalling were recorded by RU. We accompanied the manatees using a motorboat, with the motor switched off, approximately 20m from the animals, to minimise any potential stress caused to the mother and calf. The mother was a native manatee and had never been kept in captive or semi-captive acclimatisation conditions. The calf was less than a week old according to a staff at Fundação Mamíferos Aquáticos (Genilson, Personal Communication).
Mother-calf pair 2 (Aira and Bacuri)	Riacho Tabatinga, Alagoas	We recorded the vocal interactions between a female called Aira and her calf called Bacuri, from 0100h to 0309h on 1st September 2016 in the Riacho Tabatinga in the Área de Proteção Ambiental (APA) Costa dos Corais, in Porto de Pedras, Alagoas, Brazil (Figure 3.1). A total of two hours and nine minutes of uninterrupted acoustic signalling were recorded by RU. We located the mother-calf pair on the outside of one of the acclimatisation pools used for the rehabilitation and release of Antillean manatees. The observers were located on a platform above the animals, and therefore, human presence did not interfere with the recordings. Aira was most likely a re-introduced female manatee, since there was no knowledge of the occurrence of native manatees in this area. The calf was estimated to be between three and six months old by staff at the APA Costa dos Corais (Umeed Personal Communication).

Mother-calf pair 3 (Aira and new calf)	Riacho Tabatinga, Alagoas	In January 2022, we recorded Aira again with a different female calf. A total of 41.06 minutes of uninterrupted acoustic signalling were recorded, by PC for Aira alone with the new female calf. Aira and this female calf were first observed together in January 2021 (*pers. obs.* PC), suggesting that the calf was at least one year old at the time of recordings. They were recorded at a distance of 15m from the same acclimatisation pools mentioned above. But this time, the researcher was on a non-motorised raft, which is used by the *Associação Peixe*-boi during tourist visits to the acclimatisation enclosures. The mother and calf approached the raft and spent the majority of the recording time beside the raft or beneath it. At one point, the researcher entered the water to record the manatees, and during this time they approached the researcher. Thus, this scenario differs from the previous two interactions described here, as these manatees experienced close contact with humans.
Mother-calves trio (Aira, Bacuri and new calf)	Riacho Tabatinga, Alagoas	In March 2022, we recorded Aira together with her new female calf and her elder offspring, Bacuri, together. A total of 8.33 minutes of uninterrupted recordings from Aira, Bacuri and the new female calf all together were obtained by PC. The researcher recorded the animals from a tourism raft. We treated the trio as another unit in our vocalisation comparison.

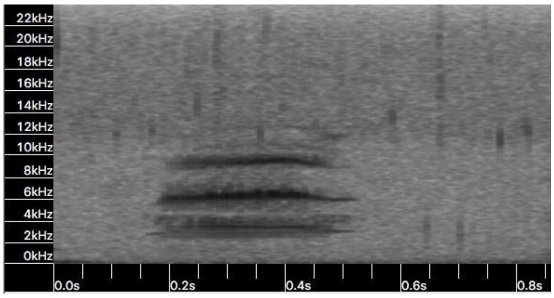
Table 3. 4. Description of the structural variables of the vocalisations included in this study. Measurements were taken from the fundamental frequency of the vocalisations.

Structural variable	**Measurement description**
Duration (ms)	Time from the start of the vocalisation to the end of the vocalisation.
Frequency of maximum energy (kHz)	Frequency at which the greatest amount of energy is found, derived from the power spectra.
Maximum and minimum frequency (kHz)	The highest and the lowest frequency of the call.
Start and end frequency (kHz)	Frequency of the beginning and the end of the call
Bandwidth (kHz)	Maximum frequency minus the minimum frequency
Distance from start to maximum frequency (ms)	Time from the beginning of the call to the point of the maximum frequency of the call
Distance from maximum frequency to the end of the call (ms)	Time from the point of maximum frequency to the end of the call
Number of harmonics	The total number of harmonics observed for each call, including subharmonics.
Inter-harmonic frequency interval (kHz)	The minimum frequency of the second harmonic minus the maximum frequency of the fundamental harmonic

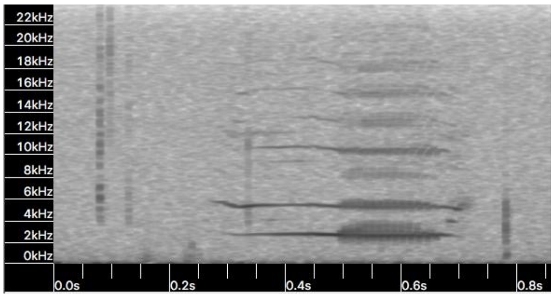
Squeak



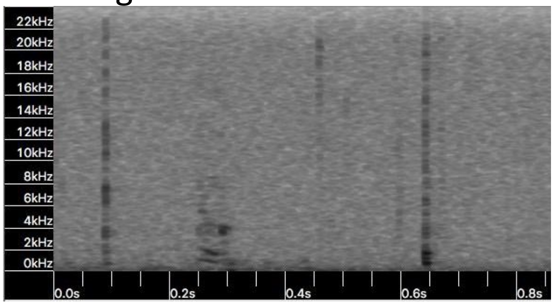
Trill



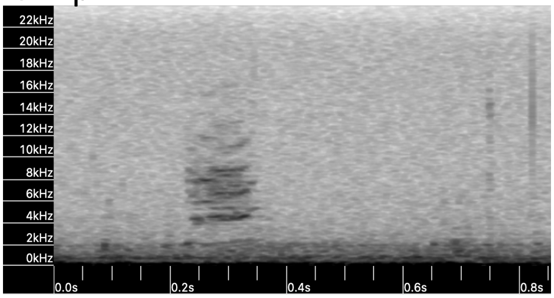
Squeak-Trill



Rubbing



Chirp



Pulse

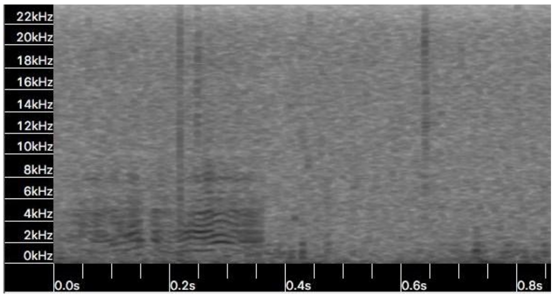


Figure 3. 2. Different types of calls considered in this study (512 samples for FFT, time resolution of 5.33ms, 50% overlap). Spectrograms created in Kaleidoscope Pro 5, Wildlife Acoustics.

4.3 Results

4.3.1 Vocalisation production

The mother-calf pair in the Mamanguape River produced a total of 54 vocalisations, categorised as Squeaks (n=46) and Trills (n=7) and Chirps (n=1) (Figure 4.3, Table 3.5), with a call rate of 0.38 calls/minute.

The mother-calf pair in the Riacho Tabatinga (Aira and Bacuri) produced a total of 163 vocalisations, categorised as Squeaks (n=85), Trills (n=47), Chirps (n=6), Pulse calls (n=15) and Rubbing (n=10) (Figure 3.2, Table 3.6), with a call rate of 1.26 calls/minute. The mother-calf pair in the Riacho Tabatinga (Aira and new female calf) produced a total of 122 vocalisations, categorised as Squeaks (n=107), Squeak-Trills (n=5), Trills (n=8), Pulses (n=2) (Figure 3.3, Table 3.5), with a call rate of 2.97 calls/minute. The trio of Aira, elder offspring Bacuri and the new calf produced, a total of 11 vocalisations categorised as Squeaks (n=8) and Squeak-Trills (n=3), with a call rate of 1.38 calls/minute.

Call descriptions are presented in Table 3.5. The structure of call types (Trill, Squeak, Squeak-trill and Rubbing) was different when considering all call parameters (PERMANOVA: Pseudo-F = 8.2234; Number of unique permutations: 9941; df = 3. 322; P = 0.0001). Pulse calls were not included in the analysis because we could only obtain duration and FME for this call type. Chirp calls were also excluded because of the small sample size available for the extraction of call variables (n=7). A Permutational t-test showed that Trills, Squeaks, Squeak-trills and Rubbing differed from each other (Table 4.31).

4.3.2 Structure of the Squeaks produced by each mother-calf pair/trio

The physical structure of the vocalisations produced by each mother-calf pair is presented in Table 3.6 and spectrograms of each call type are presented in Figure 3.4. Our DFA shows that the structure of Squeaks produced by each mother-calf pair and the trio was different, with 67.1% of cross-validated grouped cases classified correctly (Tables 3.7. and 3.8, Figure 3.5). The PERMANOVA confirmed the differences between the mother calf pairs and trio (PERMANOVA: Pseudo-F = 21.486; Number of unique permutations:

9923; df = 3, 57; P = 0.0001). Permutational t-test showed that Squeak calls differed in structure between all mother-calf pairs and trio (Table 3.9).

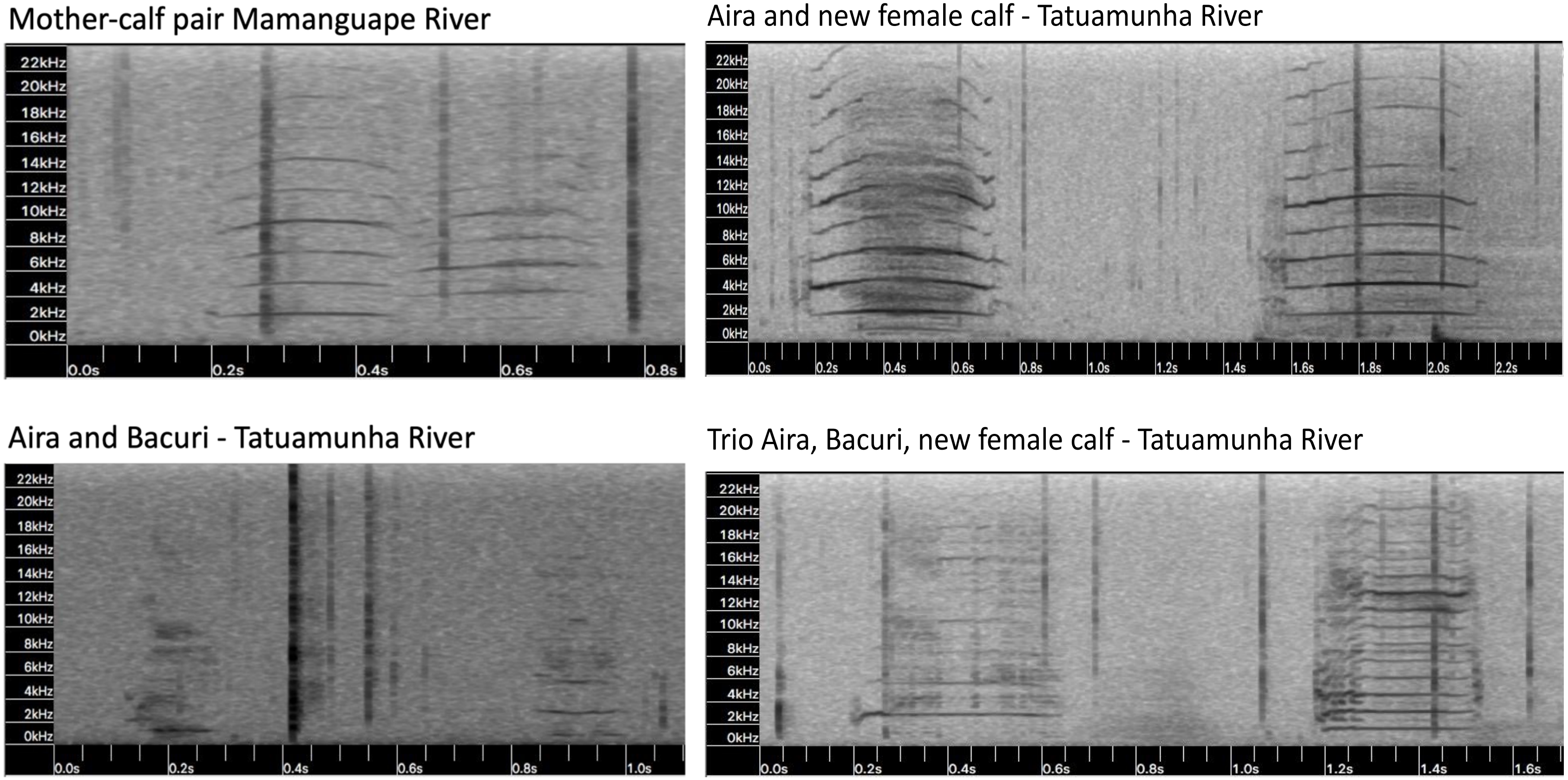
Table 3. 5. Description of the structure of the calls produced by each study mother-calf pair/trio.

Call category	**Description**
Chirps	Short (<100ms) harmonic vocalisations, see Brady et al. (2020)
Pulse	Often demonstrate a “cross-hatching” pattern, with distance between harmonics approaching the effective frequency resolution of the spectrogram (see Umeed et al., 2023).
Rubbing	Sound resulting from mechanical movement of lips rubbing on teeth (Umeed et al., 2018).
Squeak	Tonal harmonic structure, with or without the presence of subharmonic and loft regimes (Fitch et al., 2002; Mann et al., 2006; Buder et al., 2008; Umeed et al., 2018; Brady et al., 2020; Umeed et al., 2023)
Squeak-Trill	Tonal harmonic structure with the presence of bifurcations (Fitch et al., 2002; Mann et al., 2006).
Trill	Non-harmonic noisy calls with deterministic chaos (Fitch et al., 2002; Mann et al., 2006; Umeed et al., 2018).

Table 3. 6. Physical structure of the vocalisations produced by three mother-calf pairs and one mother-calf trio in North-eastern Brazil. NA indicates where values were not applicable for Pulse calls since it was not possible to take measurements from the fundamental frequency as this call did not have a tonal harmonic structure.

Mother calf pairs and Location	**Vocal sign**	**Call Duration (ms)**	**Frequency of maximum energy (kHz)**	**Maximum Frequency (kHz)**	**Minimum Frequency (kHz)**	**Bandwidth (kHz)**	**Start frequency (kHz)**	**End frequency (kHz)**	**Duration from start to peak frequency (ms)**	**Duration from peak frequency to end (ms)**	**Number of harmonics**	**Frequency interval between harmonics (kHz)**
Mother-calf pair in Mamanguape River, Paraíba	Squeak (n=46)	233.57±6.80	2.31±0.05	2.71±0.06	2.35±0.05	0.36±0.04	2.59±0.07	2.51±0.06	56.56±8.48	177.00±8.89	6.69±0.35	1.71±0.12
	Trill (n=7)	303.03±21.25	2.91±0.24	3.88±0.29	3.29±0.26	0.59±0.13	3.43±0.28	3.68±0.30	165.40±52.58	137.63±38.61	2.00±0.31	1.97±0.61
	Chirp (n=1)	99.39	2.32	2.67	2.46	0.21	2.46	2.67	40.14	59.25	6	0.79
Mother-calf pair Aira and Bacuri in Riacho Tabatinga, Alagoas	Chirp (n=6)	98.06±4.05	2.39±0.45	2.72±0.41	2.42±0.41	0.30±0.11	2.62±0.44	2.43±0.41	13.63±6.76	84.42±6.83	5.17±0.65	0.84±0.07
	Pulse (n=15)	201.89±27.71	2.57±0.34	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Rubbing (n=10)	52.76±8.21	1.05±0.09	1.53±0.13	1.12±0.09	0.41±0.07	1.22±0.09	1.44±0.14	28.22±7.10	24.54±9.10	3.80±0.28	0.24±0.70
	Squeak (n=85)	204.36±5.88	1.85±0.12	2.20±0.13	1.86±0.12	0.35±0.03	2.00±0.12	1.95±0.12	45.54±5.61	158.82±8.01	7.79±0.41	0.98±0.07
	Trill (n=47)	227.20±15.77	1.75±0.14	2.36±0.28	1.97±0.13	0.40±0.07	2.08±0.14	2.17±0.16	54.13±8.80	172.77±11.47	6.19±0.52	0.76±0.14

Mother-calf pair Aira and new female calf in Riacho Tabatinga, Alagoas	Pulse (n=2)	145.62±20.86	2.58±0.7	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Squeak (n=107)	318.48±7.71	2.33±0.08	3.14±0.09	1.99±0.08	1.15±0.07	2.87±0.09	2.68±0.0	36.29±5.55	282.61±9.61	7.36±0.45	0.99±0.11
	Squeak-Trill (n=5)	425.53± 49.62	2.55 ±0.30	3.60± 0.49	1.72 ±0.39	1.89 ±0.51	2.98 ±0.70	2.87 ±0.28	47.43 ±13.39	387.10± 58.76	4.40±1.81	0.98 ±0.11
	Trill (n=8)	291.90±29.28	2.80±0.19	3.51±0.19	2.33±0.17	1.18±0.24	3.30±0.26	3.05±0.25	17.97±9.44	273.94±26.39	3.38±0.89	0.93±0.10
Trio Aira, elder calf Bacuri, new female calf in Riacho Tabatinga, Alagoas	Squeak (n=8)	253.96±23.01	3.22±0.24	3.93±0.35	2.81±0.25	1.12±0.18	3.52±0.29	3.61±0.40	109.88±39.94	144.08±42.73	3.50±0.50	2.07±0.20
	Squeak – Trill (n=3)	334.92±18.62	2.46±0.43	3.11±0.66	2.04±0.35	1.08±0.35	2.82±0.39	2.60±0.43	33.56±38.75	301.36±56.06	4.75±0.73	2.35±0.53



Mother-calf pair Mamanguape River

Aira and new female calf - Tatuamunha River

Aira and Bacuri - Tatuamunha River

Trio Aira, Bacuri, new female calf - Tatuamunha River

Figure 3. 3. Squeak vocalisations produced by each mother-calf unit recorded in this study (i.e., mother calf par in the Mamanguape river; Aira and Bacuri, Aira and new female calf, and Trio Aira, Bacuri, new female calf in the Riacho Tabatinga). Spectrograms generated in Kaleidoscope Pro 5, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33ms, 50% overlap).

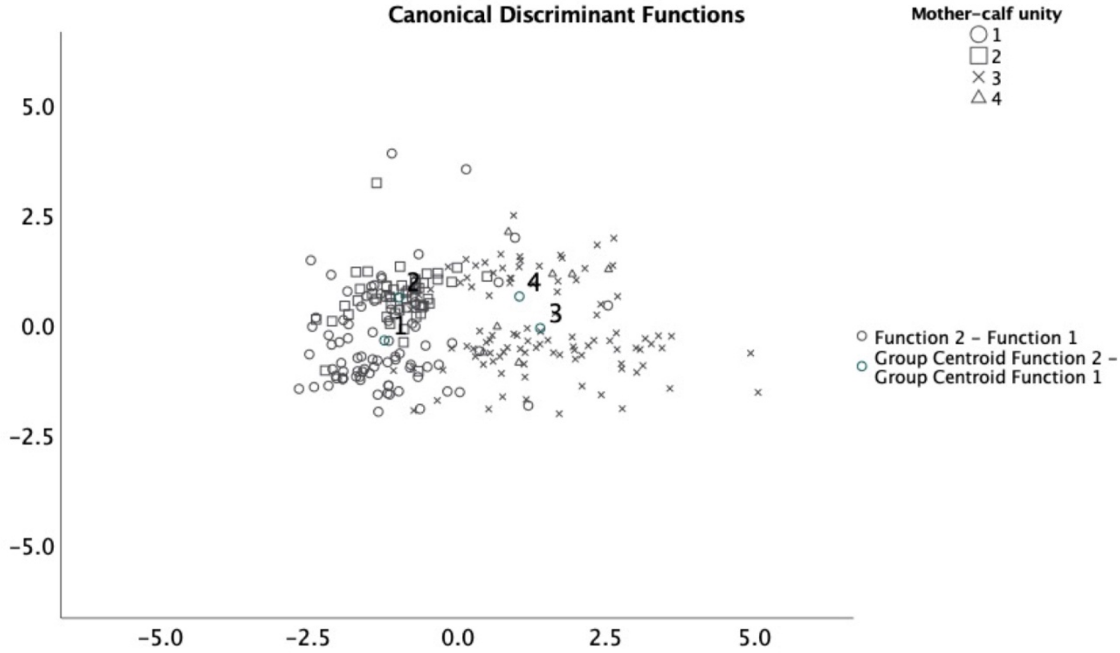


Figure 3. 4. Illustration of the canonical discriminant functions of the mother-calf pair vocalisations from each study area, with 68.7% of original grouped cases correctly classified, and 67.1% of cross-validated grouped cases correctly classified. Mother-calf units: 1) Mother calf par in the Mamanguape River; 2) Aira and Bacuri in the Riacho Tabatinga; 3) Aira and new female calf in the Riacho Tabatinga, and 4) Trio Aira/Bacuri/New female calf in the Riacho Tabatinga.

Table 3. 7. Pairwise comparisons of the structure of the call types Squeak, Trills, Squeak-trills and Rubbing produced the mother-calf units.

Call type compared	t	Number of unique permutations	P(perm)
Rubbing vs Squeak	4.0154	9944	0.0001
Rubbing vs Trill	3.3211	9948	0.0002
Rubbing vs Squeak-trill	5.9068	8922	0.0001
Squeak vs Trill	1.7693	9951	0.0284
Squeak vs Squeak-trill	2.403	9946	0.004
Trill vs Squeak-trill	2.4923	9933	0.0014

Table 3. 8. Results of the Wilk's Lambda tests carried out for each variable considered in the discriminant function analysis.

Call variables	Wilk's Lambda	F	df1	df2	p
Duration (ms)	0.610	50.894	3	239	<0.001
Frequency of maximum energy (kHz)	0.883	10.593	3	239	<0.001
Maximum frequency (kHz)	0.786	21.672	3	239	<0.001
Minimum frequency (kHz)	0.890	9.847	3	239	<0.001

Table 3. 9. Pairwise comparisons of the structure of the Squeak calls produced by each mother-calf unit: 1) Mother calf pair in the Mamanguape River; 2) Aira and Bacuri in the Riacho Tabatinga; 3) Aira and new female calf in the Riacho Tabatinga, and 4) Trio Aira/ Bacuri/New female calf in the Riacho Tabatinga.

Mother-calf unity compared	t	Number of unique permutations	P(perm)
1 vs 2	3.5008	9944	0.0001
1 vs 3	6.28	9955	0.0001
1 vs 4	3.8699	9951	0.0001
2 vs 3	4.7439	9932	0.0001
2 vs 4	3.4684	9950	0.0001
3 vs 4	2.6995	9939	0.0006

4.4 Discussion

Here we found that the mother-calf pair from the Mamanguape River produced a repertoire comprised of fewer call types (qualitatively and quantitatively) compared to the mother-calf pairs in the Riacho Tabatinga. Furthermore, call structure varied between the mother-calf pairs and trio. Thus, our prediction that vocalisations produced by the different mother-calf pairs would have different physical structures, is supported. These structural differences could be due to habitat types, infant age and size, and expected individual differences, e.g., variations in the morphology of the vocal tract (Landrau-Giovanetti et al., 2014). Currently, no such anatomical differences have been observed for West Indian manatees, *Trichechus manatus,* however research on the topic is limited (Murie, 1872; Landrau-Giovanetti et al., 2014).

Environmental variables such as pH, temperature, turbidity and salinity affect underwater sound propagation (Forrest et al. 1993; Ilyina et al. 2009; Amorim et al. 2016) and thus, the differences in call structure between mother-calf pairs could also be due to these variables in the environments where we recorded the vocalisations. The smaller body size of the Mamanguape River calf compared to the Riacho Tabatinga calves, may have resulted in the production of vocalisations with higher average maximum frequencies, as observed for infant marine mammals (e.g., Matthews et al. 1999; Sousa-Lima et al. 2002, 2008; May-Collado et al. 2007). Additionally, captive Antillean manatee calves have been found to produce vocalisations with higher values for acoustic variables (e.g., signal duration and maximum fundamental frequency) compared to adult individuals (Sousa-Lima et al. 2008) and Antillean manatee isolation calls also show some degree of structural variation (Sousa-Lima et al. 2008).

The mother-calf pair Aira and new female calf in the Riacho Tabatinga produced the highest call rate. This may be associated with the greater level of human interaction these animals experienced during recordings and may potentially indicate a higher level of excitement i.e., stress or cavorting behaviours (Brady et al., 2021). However, this finding may also be associated with calf age, since the mother-calf pair with the youngest calf (Mamanguape River) produced the lowest call rate and the mother-calf pair with the oldest calf (mother-calf pair Aira and new female calf, Riacho Tabatinga) produced the highest call rate. Nevertheless, further research is still required in order to affirm these suppositions.

We were not able to identify which vocalisations were produced by the mothers and which were produced by the calves. Notably, the mother-calf pair in the Mamanguape River (i.e., the youngest calf) produced Squeak vocalisations with the most constant maximum frequency. This may suggest that new-born/young calves imitate the frequency modulation of their mother’s calls and begin to alter the structure of their vocalisations over time. However, we suggest that further research should explore the process of vocal learning in free-living Antillean manatee calves.

Squeak call structure significantly differed between each mother-calf pair/trio. This suggests that information about individual identity is contained within these calls, which could potentially facilitate individual recognition and help to maintain contact between mothers and calves, as well as to differentiate between kin and conspecifics. We suggest the tonal vocalisations, categorised as Squeaks, may be used as potential signature vocalisations by our study free-living mother-calf pairs. Signature vocalisations are vital for maintaining contact between manatee mothers and calves (Hartman 1979; Miksis-Olds et al. 2009), especially since offspring remain with their mothers for up to two years. Thus, the use of signature vocalisations may help to prevent the stranding and/or death of new-born calves, since this is one of the main threats to the conservation of this species (Meirelles, 2008; Balensiefer et al., 2017). Notably, the mother-calf pairs produced structurally similar tonal vocalisations independent of the study habitats, i.e., estuary and mangrove. This would suggest that these types of vocalisations are most adequate for maintaining contact in dynamic manatee habitats, where variables such as pH, temperature, salinity, and turbidity are constantly changing due to tide levels.

Our study adds to the current knowledge of manatee vocal signals and may serve to help the acoustic detection of mother-calf pairs in the wild. We introduce the possibility of compiling a signature vocalisation library of free-living manatee calls to facilitate population monitoring and conservation. In order to create such a library, individual manatee calls should be recorded during eventual encounters whenever possible, as well as during veterinary check-ups. These recorded calls should be analysed by specialists and catalogued as belonging to specific manatees. Thus, during future encounters with manatees, calls can be recorded and compared with the calls stored in this library in order to identify individuals. Additionally, based on the premise that Squeak call structure contains identity information, analysing the call structures of vocalisations collected, either using passive or active acoustic monitoring, could be used together with other surveying methods (e.g., drone, sonar etc.) to estimate the population abundance of manatees in North-eastern Brazil. The use of recorded calls may also be interesting with regards to reuniting separated mothers and calves, considering that calf strandings due to separation is unfortunately a common occurrence in certain regions in North-eastern Brazil (see for instance, Balensiefer et al., 2017). Calf isolation calls could be recorded during stranding events and played back *in situ* in order to attract the mother to the location of the calf. Thus, it is important to establish partnerships with local organisations and academic institutions in order to advance monitoring and surveying strategies.

5 Article 3 - Tidal and diel influence on the Antillean manatee, *Trichechus manatus manatus*, vocalisations in North-eastern Brazil

5.1 Introduction

Acoustic communication plays an important role in the survival of individuals and is particularly important in social contexts, individual recognition, reproduction and maintaining contact with conspecifics (Dall et al., 2005; Sousa-Lima et al., 2008; Opzeeland, 2010; López, 2022). The vocal communication of animals may be directly or indirectly influenced by the characteristics of their environment (Leão et al., 2016). Factors such as seasonality, time period and tide level have been shown to influence the call production patterns of many species of marine mammals, such as Southern right whales, *Eubalaena australis* (Shabangu et al., 2021); fin whales, *Balaenoptera physalus* (Simon et al., 2010); blue whales, *Balaenoptera musculus* (Wiggins et al., 2005); sei whales, *Balaenoptera borealis* (Baumgartner & Fratantoni, 2008); common bottlenose dolphin, *Tursiops truncatus* (Kremers et al., 2014; Monczak et al., 2019; Marian et al., 2021; Probert et al., 2021); Indo-Pacific humpback dolphins, *Sousa chinensis* (Lin et al., 2013); dugongs, *Dugong dugon* (Tanaka et al., 2016); Amazonian River dolphins, *Inia geoffrensis* (Amorim et al., 2016); humpback whales, *Megaptera novaeangliae* (Au et al., 2000; Barlow et al., 2019), and short-beaked common dolphins, *Delphinis delphus* (Goold et al., 2000).

Differences in call rate throughout the day and during different tide regimes have been found for many species of marine mammals. For example, Shabangu et al. (2020) observed an increase in vocal activity of Southern right whales in the early predawn hours and from the afternoon to midnight, during the spring, with a decrease in vocal activity at mid-day. Blue whales have also been found to produce more calls during the summer and autumn periods (Wiggins et al., 2005). During transitions from low-to-high and high-to-low tides, their call rates were correlated with sunset and sunrise~~,~~ respectively (Wiggins et al., 2005). Furthermore, higher vocalisation rates during the day have also been observed for sei whales (Baumgartner & Fratantoni, 2008). Rescued Florida manatees, *Trichechus manatus latirostris*, demonstrated significant differences in diel vocal patterns, presenting higher vocalisation rates in the AM compared to PM (Civelek, 2013). Whereas African manatees, *Trichechus senegalensis*, were found to produce the most vocalisations during the middle of the night and at dusk (Rycyck et al., 2021).

Environments exert some level of selective pressure which results in the selection of the fittest individuals and evolutionarily selected adaptations to specific acoustic contexts (Podos et al., 2004; Leão et al., 2016). However, much is still to be discovered in relation to the diurnal vocal patterns and the influence of tide level on the vocal production of the Endangered Antillean manatee, *Trichechus manatus manatus*. This sub-species inhabits estuarine environments in North-eastern Brazil and is restricted to shallow and warm waters. Thus, to survive, manatees must withstand the daily routine changes associated with such environments (Lin et al., 2013). Estuaries are strongly influenced by tidal regimes, which affect water depth and turbidity through the mixing and stratification of fresh and saltwater (Lin et al., 2013). Thus, to understand the role and importance of manatee vocal communication, it is important to investigate the environmental factors that may influence call production and structure (Tanaka et al., 2016).

Several studies have detailed the vocal production of Antillean manatees, describing calls with fundamental frequencies ranging from 0.64 to 5.23 kHz (O'Shea & Poché, 2006; Umeed et al., 2018; Merchan et al., 2019, Umeed et al., 2023), with new findings indicating harmonic frequencies reaching up to 150kHz (Ramos et al., 2020). The vocal repertoire of Antillean manatees is comprised of five calls: Squeaks, Trills, Chirps, Pulses and Rubbing calls, all of which present differences in call structure and production according to sex and age (Sousa-Lima et al., 2008; Umeed et al., 2018; Umeed et al., 2023). Studies have also suggested the production of calls containing individual information, which likely facilitates individual recognition and caller identity (Sousa-Lima et al., 2008; Umeed et al., 2023; Umeed chapters 3.1 and 3.2 of this thesis).

Little is known about diel and tidal influence on the vocal patterns of Antillean manatees. However, Umeed et al. (2018; 2023) showed that manatees housed in captive and reintroduction conditions presented differences in call structure according to sex, age and housing condition. Thus, we know that manatees alter their vocal production to produce calls with different structures at some point in their lives. Therefore, since manatees present some level of vocal complexity, we would expect that they would also adjust their vocal structure and production according to fluctuations in their environment. Studies have shown that the structure of Amazon River dolphins, *Inia geoffrensis*, pulses differ structurally according to water turbidity, where dolphins produced calls with significantly higher maximum frequencies, centre frequency bandwidths and number of harmonics in more turbid water (Amorim et al., 2016). Furthermore, Deconto & Monteiro-Filho (2015) found that Guiana dolphin, *Sotalia* *guianensis*, whistles differed structurally throughout the day, presenting lower frequency sounds during the night.

Here, we aimed to investigate the influence of diel period (as a proxy of temperature and illumination period) and tide level (as a proxy of pH, temperature and salinity) on the call production and structure of Antillean manatees housed in captive and natural reintroduction conditions. We expected animals to produce more vocalisations in situations of limited visibility (i.e., during the night and peak low tide) and an overall higher production of tonal harmonic vocalisations (described here as Squeaks following Umeed et al., 2018, Umeed et al., 2023) during all diel periods and tidal regimes, compared to other types of vocalisations. We also expect the structure of Antillean manatee calls to differ according to day period and tide level, as an adaptation to their constant changing estuarine environment.

5.2 Material and Methods

5.2.1 Study areas and animals

Between September 2018 and July 2019, 14 manatees were monitored in three locations. This study was performed in three locations: the Instituto Chico Mendes de Conservação da Biodiversidade/ Centro Nacional de Pesquisa e Conservação de Mamíferos Aquaticos (ICMBio/CMA), on Itamaracá Island – PE. The second site was the Centro de Reintrodução da Área de Proteção Ambiental (APA) (Environmental Protection Area) Costa dos Corais, Porto de Pedras – AL and the third site was the Recinto Jocélio de Brito in the APA Barra do Rio Mamanguape - PB (Figure 4.1).

Figure 4. 1. Map of the study locations (source: Google Earth) and a) the two reintroduction pools in APA Costa dos Corais, AL; b) the two captive pools at the ICMBio/CMA, PE and c) the Recinto Jocélio de Brito reintroduction pool, PB (photos: Authors)

A total of six captive females, four captive males, four reintroduction males in Alagoas, and a reintroduction pair in Paraíba (one male and one female) were recorded during this study (Table 4.1).

Table 4. 1. Characteristics of the individuals recorded during this study. *Vitória and Parajuru were translocated from the ICMBio/CMA, PE to the Recinto Jocélio Brito, PB in April 2019. PE: Pernambuco; PB: Paraíba; AL: Alagoas.

Individual	**Sex**	**Estimated age (yrs.)**	**Age Category**	**Reintroduction/Captivity Centre**	**Location**
Bela	Female	8	Adult	ICMBio/CMA	PE
Canoa	Female	14	Adult	ICMBio/CMA	PE
Carla	Female	22	Adult	ICMBio/CMA	PE
Paty	Female	4	Juvenile	ICMBio/CMA	PE
Sheila	Female	22	Adult	ICMBio/CMA	PE
Xuxa	Female	32	Adult	ICMBio/CMA	PE
Vitória*	Female	4.5	Juvenile	ICMBio/CMA/reintroduction centre APA Barra do Rio Mamanguape	PE/PB
Daniel	Male	9	Adult	ICMBio/CMA	PE
Parajuru*	Male	6	Adult	ICMBio/CMA/reintroduction centre APA Barra do Rio Mamanguape	PE/PB
Poque	Male	27	Adult	ICMBio/CMA	PE
Zoé	Male	14	Adult	ICMBio/CMA	PE
Assu	Male	20	Adult	Reintroduction centre APA Costa dos Corais	AL
Netuno	Male	28	Adult	Reintroduction centre APA Costa dos Corais	AL
Aratí	Male	9	Adult	Reintroduction centre APA Costa dos Corais	AL
Raimundo	Male	8	Adult	Reintroduction centre APA Costa dos Corais	AL

At the ICMBio/CMA, the four males and six females were kept in separate pools with dimensions 8m x 5m and 4m deep and 10 m x 5 m, and 4 m deep, respectively. These pools are constructed from concrete and fibre glass (Figure 4.1). At the APA Costa dos Corais, the four animals were divided into two reintroduction pools, with one housing two younger males (Aratí and Raimundo, known herein as younger reintroduction males-AL) and the other housing two older males (Netuno and Assu, known herein as older reintroduction males-AL). The dimensions of the younger male pool were 31m x 17m and the older male pool 27 m x 13 m, with the depth of each pool varying between <1m and 2.5m depending on the tide level (Figure 4.1). These pools are located on the Riacho Tabatinga, Alagoas State, Brazil, in a mangrove area and do not have fixed walls, rather the boundaries of the pools are marked with wooden pools secured into the ground. Thus, material can enter and leave the pools and the animals are able to make direct physical contact with manatees outside the pools by touching flippers or snouts. Furthermore, these pools experience the effects of changes in tide levels. The sedimentation of the Tatuamunha River estuary, from which the Riacho Tabatinga branches, is sandy lithoclastic and acts as a potential retainer of organic matter and terrigenous mud, from the middle to upper estuary (Paiva, 2018).

Two manatees, Vitória and Parajuru (known herein as reintroduction pair-PB), were translocated from the ICMBio/CMA to the reintroduction pool in Barra de Mamanguape, Paraíba in April 2019. Thus, these animals were included in both the captive male and female data and the reintroduction pair data. Notably, neither Vitória nor Parajuru had previous contact with the opposite sex before their translocation and both animals were rescued as stranded calves and therefore had no experience of natural estuarine conditions. The Recinto Jocélio de Brito (RJB) is located in the Gamboa de Caracubu, where the Caracabu River meets the Mamanguape River, also in a mangrove area (Figure 4.1). The sedimentation of the Gamboa de Caracabu is sandy-muddy, comprised of pieces of shells, gravel, sand and mud (Alencar, 2010)

5.2.2 Vocalisation Recordings

We recorded the vocalisations continuously (24 hours per day) using a SM4 Wildlife Acoustics recorder attached to SM3 hydrophonex hydrophone (Wildlife Acoustics), i.e., vocalisations were recorded continually for 24 hours each day. A total of 316 hours were recorded and analysed at the three study sites. At the ICMBio/CMA and APA Costa dos Corais reintroduction centre, the hydrophone was placed inside a pipe in order to avoid possible damage to the hydrophone and also to the manatees, particularly during the night. The pipe was not required in the Recinto Jocélio Brito.

5.2.3 Environmental variables

In this study, we considered the following environmental variables: time of the day (as a proxy of temperature and illumination period) and tide level (to indirectly account for pH, temperature, salinity and turbidity). The hours of the day were divided into six sub-periods: 1am-5am, 5am-9am, 9am-1pm, 1pm-5pm, 5pm-9pm, 9pm-1am. The tide levels were divided into four categories: ebb (period between the high tide and low), peak low (peak low tide), flood (period between the low and high tide) and peak high (peak high tide). Tides were calculated using the tide tables for Maceio Port, Alagoas and for Cabedelo Port, Paraíba (Table 4.2).

Table 4. 2. Tide levels and sunrise/sunset times for each of the study days in the APA Costa dos Corais, Alagoas, Brazil and the APA Barra do Rio Mamanguape, Paraíba, Brazil. Tide levels were obtained from tide tables for Maceio Port and Cabedelo Port.

Location	**Date**	**Peak high tide (m)**	**Time of day**	**Peak low tide**	**Time of day**	**Sunrise (am)**	**Sunset (pm)**
APA Costa dos Corais, Alagoas, Brazil	06/02/2019	2	04:39am	0.3	10:45am	4:22	4:51
		2.1	04:47pm	0.3	11:02pm		
	07/02/2019	2	05:09am	0.4	11:13am	4:22	4:51
		2.1	05:19pm	0.3	11:24pm		
	08/02/2019	1.9	05:43am	0.4	11.47am	4:23	4:50

		2.0	5:54pm				
	09/02/2019	1.9	06:15am	0.4	0:04am	4:23	4:50
		1.9	06:30pm	0.5	12:19pm		
	10/02/2019	1.8	06:54am	0.5	0:41am	4:23	4:50
		1.8	7:09pm	0.6	12:56pm		
	11/02/2019	1.7	07:38am	0.6	01:21am	4:23	4:50
		1.7	8:00pm	0.6	01:43pm		
	12/02/2019	1.6	08:23am	0.7	02:11am	4:24	4:50
		1.6	09:06pm	0.7	02:43pm		
APA Barra do Rio Mamanguape, Paraíba	18/04/2019	2.5	03:34am	0.1	09:34am	05:21	05:16
		2.6	03.53pm	0.0	09:59pm		
	19/04/2019	2.6	04:13am	0.0	10:13am	05:21	05:15
		2.6	04:36pm	0.0	10:38pm		
	20/04/2019	2.6	04:54am	0.0	10:56am	05:21	05:14
		2.6	05:15pm	0.1	11.17pm		
	21/04/2019	2.5	05:32am	0.1	11:36am	05:21	05:14
		2.5	05:56pm	0.3	11:56pm		
	22/04/2019	2.4	06:09am	0.3	12:25pm	05:21	05:14
		2.3	06:38pm				
	23/04/2019	2.3	06:49am	0.5	00:32am	05:21	05:13
		2.1	07:19pm	0.4	12:58pm		
	24/04/2019	2.1	07:30am	0.7	01:09am	05:21	05:13
		1.9	08:06pm	0.6	01:43pm		
	25/09/2019	1.8	08:21am	0.8	01:56am	05:21	05:13
		1.7	09:21pm	0.8	02:43pm		

5.2.4 Analysis of vocalisation production and pattern

The vocalisations were categorised based on Umeed et al. (2018), Umeed et al., 2023 and chapter 3.1 of this thesis: Squeaks, Trills, Chirps, Pulses and Rubbing. In order to identify patterns of vocalisation production and vocalisation types, we used Kaleidoscope Pro 5, Wildlife Acoustics software. The average call rate (i.e., total number of calls/the number of animals/the number of hours) and the types of vocalisations produced were quantified for each site. We also analysed the structure of 10 Squeak calls produced during each time sub-period and tide level using the same analysis software mentioned above. We selected four structural variables: duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz). Squeak calls were selected as they were produced by all study groups and were the most commonly produced call by all study groups.

We used Chi-squared analyses to test for differences in call rates according to time of the day and tide levels both between and within groups. We also used Chi-squared tests to identify differences in the types of calls produced according to time of day and tide level. We performed Discriminant Function Analyses, on SPSS v29, to verify whether the structures of calls produced during different times of the day and tide levels differed significantly and whether they could be assigned to each condition. Tide level comparisons were only considered for the reintroduction animals (PB and AL) since this factor was not applicable to the captive study animals.

5.3 Results

5.3.1 Overall call production according to time of day and tide level

We analysed a total of 28,202 vocalisations from all five groups of animals, of which a total of 3393 were produced by the captive males (42 hours), 9799 were produced by the captive females (52 hours), 5552 were produced by the reintroduction pair at PB (111 hours), 6916 were produced by the older reintroduction males at AL (70 hours), and 4433 calls were produced by the younger reintroduction males at AL (41 hours) (Figure 4.2).

The average call rate of each manatee group (i.e., number of calls/number of manatees/number of hours) differed significantly for the time periods of 5am-9am ($X^2=10.908$, d.f.=4, $p<0.05$), 9am-1pm ($X^2=40.036$, d.f.=4, $p<0.05$), 1pm-5pm ($X^2=20.939$, d.f.=4, $p<0.05$) and 5pm-9pm ($X^2=42.409$, d.f.=4, $p<0.05$). Significant differences were not found for the time periods of 9pm-1am and 1am-5am ($X^2=3.745$, d.f.=4, $p>0.05$; $X^2=9.699$, d.f.=4, $p>0.05$, respectively).

We also found that the call rates of the manatee groups (reintroduction pair-PB, older reintroduction males-AL and younger reintroduction males-AL) differed significantly for all tide levels: ebb: $X^2=57.092$, d.f.=2, $p<0.05$; peak low: $X^2=44.643$, d.f.=2, $p<0.05$; flood: $X^2=51.713$, d.f.=2, $p<0.05$ and peak high: $X^2=87.208$, d.f.=2, $p<0.05$.

The captive males and older and younger reintroduction males-AL produced all five different call types. The reintroduction pair-PB produced four call types: Squeaks, Trills, Chirps and Pulses and captive females only produced two call types: Squeaks and Trills (Figure 4.2).

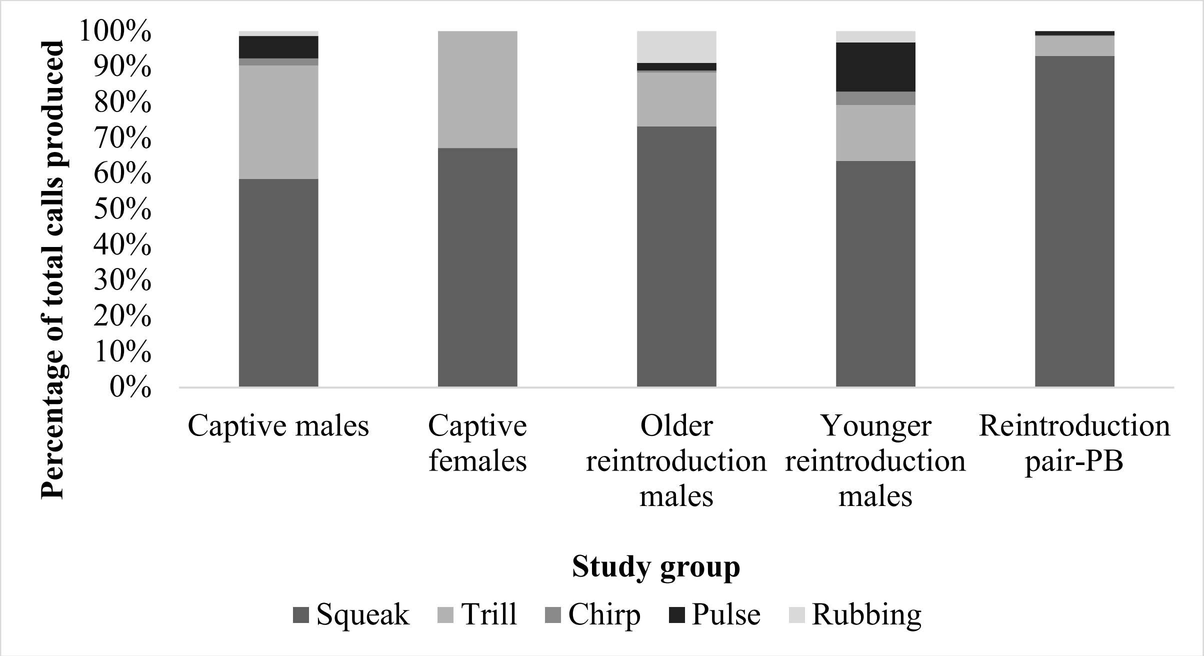


Figure 4. 2. Overall proportion (%) of call types produced by each study group: Captive males; Captive females; Older reintroduction males-AL; Younger reintroduction males-AL and Reintroduction pair-PB.

5.3.2 Call structure and production according to time of day and tide level

5.3.2.1 Captive males and females

We found that the average captive male call rate was highest between 5am-9am (n=50.5 calls/hour) and lowest between 5pm and 9pm (n=10.688 calls/hour). Captive male call production differed significantly depending on time of day ($X^2=37.278$, d.f.=5, $p<0.05$). The highest average call rate of the captive females was also between 5am-9am (n=50.542 calls/hour) and the lowest call rate was between 9am-1pm (n=15.011 calls/hour). Captive female call rate differed significantly depending on time of day ($X^2=19.357$, d.f.=5, $p<0.05$).

The call rates of each call type produced by captive males were significantly different for all time sub-periods, considering all call categories (Squeaks, Trills, Chirps, Pulses, Rubbing), where they produced significantly more Squeak calls compared to the other call types ($p<0.05$) (Table 4.3). However, the call rates of each call type produced by captive females were not significantly different. Furthermore, the Squeak call rate differed depending on the time sub-period for both males ($X^2=37.567$, d.f.=5, $p<0.05$) and females ($X^2=16.375$, d.f.=5, $p<0.05$).

Only one out of four call parameters contributed to the differentiation of calls (Table 4.5) when analysing the structure of Squeak calls produced by captive males throughout the day (Table 4.4) and only 38% of correct call classification was achieved. All analysed squeak call parameters varied during different times of day for Squeaks produced by captive females (Table 4.6) with 56.7% of calls correctly assigned to time of day.

Table 4. 3. Chi-squared values, with Yate's correction for the captive females, for the call rates of each call type during each time sub-period.

Manatee group	**Time sub-period**	**X^2**	**Degrees of freedom**	**p-value**
Captive males	1am-5am	54.000	4	P<0.05
	5am-9am	74.407	4	P<0.05
	9am-1pm	19.660	4	P<0.05
	1pm-5pm	18.390	4	P<0.05
	5pm-9pm	12.406	4	P<0.05
	9pm-1am	31.153	4	P<0.05
Captive females	1am-5am	0.052	1	P>0.05
	5am-9am	0.663	1	P>0.05
	9am-1pm	1.548	1	P>0.05
	1pm-5pm	0.186	1	P>0.05
	5pm-9pm	0.257	1	P>0.05
	9pm-1am	0.521	1	P>0.05

Table 4. 4. Average duration(ms), frequency of maximum energy (kHz), maximum frequency (kHz), minimum frequency (kHz) and bandwidth (kHz) for Squeak calls produced by captive males and females throughout the day.

Manatee group	**Time of day**	**Duration (ms)**	**Frequency of maximum energy (kHz)**	**Maximum frequency (kHz)**	**Minimum frequency (kHz)**	**Bandwidth (kHz)**
Captive males	1am-5am	296.559±15.012	3.385±0.232	3.629±0.190	2.7085±0.181	0.921±0.191
	9am-1pm	148.854±6.803	3.167±0.325	3.583±0.333	2.901±0.317	0.682±0.055
	1pm-5pm	239.490±20.409	2.930±0.051	3.335±0.111	2.593±0.150	0.742±0.066
	5pm-9pm	292.581±12.621	2.627±0.044	3.075±0.055	2.352±0.063	0.723±0.066
	9pm-1am	272.651±26.451	2.753±0.120	3.219±0.113	2.545±0.112	0.675±0.080
Captive females	1am-5am	378.586±25.323	2.517±0.135	3.152±0.198	2.236±0.117	0.916±0.105
	5am-9am	429.444±8.628	2.923±0.046	3.6337±0.081	2.483±0.067	1.150±0.071
	9am-1pm	347.576±24.712	1.993±0.140	2.485±0.130	1.744±0.113	0.741±0.032
	1pm-5pm	496.178±19.489	1.985±0.013	2.631±0.046	1.811±0.031	0.815±0.056
	5pm-9pm	304.166±19.307	2.527±0.141	2.968±0.153	2.303±0.130	0.665±0.055
	9pm-1am	384.824±10.814	2.721±0.078	3.363±0.106	2.522±0.0751	0.841±0.065

Table 4. 5. Contribution of squeak call parameters: Syllable duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz) to the differentiation of calls produced during different hours of the day by captive males (n=4).

Squeak call parameter	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Duration (ms)	0.484	11.986	4	45	<0.001
Frequency of maximum energy	0.919	0.990	4	45	0.423
Maximum Frequency	0.876	1.597	4	45	0.191
Minimum Frequency	0.904	1.199	4	45	0.324

Table 4. 6. Contribution of squeak call parameters: Syllable duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz) to the differentiation of the structure of calls produced during different hours of the day by captive females (n=6).

Squeak call parameter	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Duration (ms)	0.472	12.105	5	54	<0.001
Frequency of maximum energy	0.448	13.330	5	54	<0.001
Maximum Frequency	0.485	11.485	5	54	<0.001
Minimum Frequency	0.469	12.225	5	54	<0.001

5.3.2.2 Reintroduction pair - PB

The reintroduction pair-PB presented the highest average call rate between 1am-5am (n=57.3 calls/hour) and the lowest average call rate between 9am-1pm (n=10.478 calls/hour). The highest average call rate of the pair was during the peak low tide (n=37.056) calls/hour and the lowest during the peak high tide (n=28.143 calls/hour). Call production was relatively consistent, independent of time period and tide level ($p>0.05$), i.e., call production was relatively consistent independent of time period and tide level. The call rate of each specific call type was significantly different for all time sub-periods considering all call categories, where the animals produced significantly more Squeak calls compared to the other call types ($p<0.05$) (Table 4.7). Call rates for specific call types were significantly different for all tide levels, with manatees producing significantly more Squeak vocalisations compared to the other call types (Table 4.7). Squeak call rates differed depending on time sub-period (X^2=64.456, d.f.=5, p<0.05) but not tide level.

Calls produced during the day varied for three out four squeak call parameters (Tables 4.8, 4.9), however they did not vary enough to provide a strong correct call classification as only 26.3% of the squeak calls were correctly classified, considering leave one out cross-validation method. This was also observed when considering squeak calls produced at different tide levels (Table 4.10). Approximately 41.4% of the squeak calls were correctly assigned to the different tide levels.

Table 4. 7.Chi-squared values for the average overall call rate for the reintroduction pair-PB for each time sub-period and tide level.

Manatee group	**Time sub-period/tide level**	**X^2**	**Degrees of freedom**	**p-value**
Reintroduction pair-PB	1am-5am	197.777	4	P<0.05
	5am-9am	84.451	4	P<0.05
	9am-1pm	32.144	4	P<0.05
	1pm-5pm	46.900	4	P<0.05
	5pm-9pm	41.786	4	P<0.05
	9pm-1am	57.857	4	P<0.05
	Flood	106.198	4	P<0.05
	Peak high	105.610	4	P<0.05
	Ebb	96.914	4	P<0.05
	Peak low	125.255	4	P<0.05

Table 4. 8. Average duration(ms), frequency of maximum energy (kHz), maximum frequency (kHz), minimum frequency (kHz) and bandwidth (kHz) for Squeak calls produced by the reintroduction pair-PB throughout the day and tide regimes.

Manatee group	Time of day	Tide level	Duration (ms)	Frequency of maximum energy (kHz)	Maximum frequency (kHz)	Minimum frequency (kHz)	Bandwidth (kHz)
Reintroduction pair-PB	1am-5am	flood	228.912±12.734	2.137±0.026	2.452±0.031	2.026±0.037	0.426±0.051
	1am-5am	ebb	283.691±12.956	1.964±0.051	2.305±0.055	1.765±0.077	0.539±0.089
	1am-5am	peak high	291.238±7.292	2.426±0.098	2.383±0.039	1.861±0.054	0.522±0.057
	1am-5am	peak low	249.107±12.457	2.093±0.033	2.554±0.038	1.873±0.030	0.680±0.016
	5am-9am	peak high	336.031±19.988	1.908±0.029	2.336±0.072	1.689±0.037	0.646±0.082
x	9am-1pm	flood	256.902±19.268	2.063±0.036	2.452±0.044	1.797±0.052	0.655±0.044
	9am-1pm	ebb	272.211±18.267	2.044±0.051	2.444±0.044	1.852±0.058	0.592±0.038
	9am-1pm	peak low	291.025±9.804	1.941±0.048	2.209±0.052	1.748±0.057	0.461±0.047
	1pm-5pm	flood	267.746±8.477	2.149±0.039	2.444±0.048	1.957±0.049	0.487±0.032
	1pm-5pm	ebb	334.817±14.272	2.167±0.045	2.574±0.086	2.014±0.072	0.560±0.062
	1pm-5pm	peak low	226.868±8.570	3.004±0.348	3.339±0.329	2.852±0.329	0.487±0.037
	5pm-9pm	flood	256.685±16.653	2.741±0.336	3.140±0.336	2.513±0.304	0.627±0.075
	5pm-9pm	ebb	230.864±10.113	1.970±0.053	2.322±0.054	1.826±0.070	0.496±0.047
	5pm-9pm	Peak low	271.725±12.377	2.019±0.055	2.367±0.045	1.751±0.041	0.615±0.040
	9pm-1am	flood	214.442±8.639	2.065±0.019	2.602±0.058	1.913±0.019	0.689±0.066
	9pm-1am	ebb	298.572±13.150	1.900±0.034	2.357±0.038	1.609±0.063	0.748±0.043
	9pm-1am	peak low	270.202±7.485	1.855±0.021	2.252±0.024	1.572±0.043	0.680±0.051

Table 4. 9. Contribution of squeak call parameters: Syllable duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz) to the differentiation of calls produced during different hours of the day by the reintroduction pair-PB (n=2).

Squeak call parameter	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Duration (ms)	0.866	5.548	5	180	<0.001
Frequency of maximum energy	0.981	.696	5	180	0.627
Maximum Frequency	0.905	3.767	5	180	0.003
Minimum Frequency	0.847	6.493	5	180	<0.001

Table 4. 10. Contribution of squeak call parameters: Syllable duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz) to the differentiation of calls produced during different tide levels by the reintroduction pair-PB (n=2).

Squeak call parameter	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Duration (ms)	0.810	14.193	3	182	<0.001
Frequency of maximum energy	0.969	1.968	3	182	0.120
Maximum Frequency	0.955	2.840	3	182	0.039
Minimum Frequency	0.954	2.901	3	182	0.036

5.3.2.3 Older reintroduction males-AL

The highest average call rate for the older reintroduction males was between 5pm-9pm (n=77.955 calls/hour) and during the flood tide (n=60.333 calls/hour) and the lowest between 9pm-1am (n=21.964 calls/hour) and during peak low tide (n=21.167 calls/hour). We found that call rate did not vary significantly depending on the time of day (p>0.05), however it varied significantly according to tide level (X^2=30.762, d.f.=3, p<0.05). Call rates of specific call types were significantly different for all time sub-periods considering all call categories, where the animals produced significantly more Squeak calls compared to the other call types (p<0.05) (Table 4.1-11). Call rate according to call type was significant for all tide levels, with manatees producing significantly more Squeak vocalisations compared to the other call types (Table 4.11). Furthermore, Squeak call rate differed depending on time sub-period (X^2=21.129, d.f.=5, $p<0.05$) and tide level (X^2=23.233, d.f.=3, $p<0.05$).

Three out four squeak call parameters (Table 4.12) varied during different times of the day (Table 4.13) but, they did not vary enough to provide a strong correct call classification as only 31% of the squeak calls were correctly classified considering a leave one out cross validation method. The same was observed when considering squeak calls produced at different tide levels (Table 4.13). Approximately 51% of the squeak calls were correctly assigned to the different tide levels.

Table 4. 11. Chi-squared values for the number of calls produced per older reintroduction-AL manatee per hour for each time sub-period and tide level.

Manatee group	**Time sub-period/tide level**	**X^2**	**Degrees of freedom**	**p-value**
Older reintroduction males-AL	1am-5am	112.449	4	P<0.05
	5am-9am	117.337	4	P<0.05
	9am-1pm	161.612	4	P<0.05
	1pm-5pm	141.674	4	P<0.05
	5pm-9pm	70.596	4	P<0.05
	9pm-1am	40.499	4	P<0.05
	Flood	136.828	4	P<0.05
	Peak high	52.485	4	P<0.05
	Ebb	122.976	4	P<0.05
	Peak low	47.387	4	P<0.05

Table 4. 12. Average duration(ms), frequency of maximum energy (kHz), maximum frequency (kHz), minimum frequency (kHz) and bandwidth (kHz) for Squeak calls produced by older reintroduction males throughout the day and tide regimes.

Manatee group	**Time of day**	**Tide level**	**Duration (ms)**	**Frequency of maximum energy (kHz)**	**Maximum frequency (kHz)**	**minimum frequency (kHz)**	**Bandwidth (kHz)**
Older reintroduction males	1am-5am	flood	223.458±20.876	4.368±0.187	5.076±0.232	3.995±0.216	1.081±0.167
	1am-5am	peak low	311.108±15.749	2.466±0.185	2.930±0.268	2.227±0.140	0.704±0.139
	5am-9am	flood	266.001±17.858	3.810±0.284	4.405±0.331	3.225±0.248	1.180±0.214
	5am-9am	ebb	271.559±16.252	2.425±0.178	2.955±0.201	2.265±0.187	0.690±0.091
	5am-9am	peak high	274.044±19.929	2.684±0.251	3.152±0.251	2.332±0.216	0.820±0.066
	9am-1pm	flood	227.741±12.890	3.790±0.069	4.415±0.078	3.383±0.127	1.031±0.159
	9am-1pm	ebb	414.350±8.842	2.364±0.212	2.774±0.144	1.874±0.040	0.899±0.154
	9am-1pm	peak low	333.159±14.565	2.641±0.343	2.814±0.276	1.928±0.143	0.887±0.215
	1pm-5pm	flood	305.299±13.497	3.476±0.401	3.866±0.416	3.298±0.389	0.568±0.059
	1pm-5pm	peak low	367.335±11.440	35.562±33.205	2.853±0.165	2.072±0.022	0.781±0.162
	5pm-9pm	flood	267.832±10.182	3.627±0.309	4.425±0.372	2.882±0.272	1.543±0.208
	5pm-9pm	ebb	295.692±20.539	26.147±23.093	3.720±0.299	2.824±0.186	0.896±0.142
	5pm-9pm	peak high	309.084±30.186	2.468±0.100	2.953±0.122	2.147±0.077	0.806±0.118
	9pm-1am	ebb	269.021±13.866	3.035±0.128	3.653±0.175	2.665±0.156	0.988±0.100
	9pm-1am	peak low	314.006±17.358	2.439±0.163	2.750±0.182	2.236±0.160	0.513±0.056

Table 4. 13. Contribution of squeak call parameters: Syllable duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz) to differentiate calls uttered under different hours of the day by older reintroduction males.

Squeak call parameter	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Duration (ms)	0.853	5.228	5	152	<0.001
Frequency of maximum energy	0.964	1.121	5	152	0.352
Maximum Frequency	0.857	5.070	5	152	<0.001
Minimum Frequency	0.819	6.712	5	152	<0.001

Table 4. 14. Contribution of squeak call parameters: Syllable duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz) to differentiate calls uttered under different tide regimes by older reintroduction males.

Call parameter	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Duration (ms)	0.855	8.707	3	154	<0.001
Frequency of maximum energy	0.991	0.475	3	154	0.700
Maximum Frequency	0.635	29.495	3	154	<0.001
Minimum Frequency	0.644	28.346	3	154	<0.001

5.3.2.4 Younger reintroduction males–AL

The younger reintroduction males-AL produced the highest average call rate between 9am-1pm (n=73.278 calls/hour) and the lowest call rate between 9pm-1am (n=24.850 calls/hour). They also presented the highest call rate during peak high tide (n=71 calls/hour) and the lowest call rate during peak low tide (n=47.357 calls/hour). The overall call production of the younger reintroduction males differed significantly depending on time of day (X^2=15909, d.f.=5, $p<0.05$), however it did not differ significantly according to tide level ($p>0.05$). Call rate according to call type was significantly different for all time sub-periods considering all call categories, where the animals produced significantly more Squeak calls compared to the other call types ($p<0.05$) (Table 4.15). Call rate according to call type was also significantly different for all tide levels, with manatees producing significantly more Squeak vocalisations compared to the other call types (Table 4.15). Furthermore, Squeak call rate differed depending on time sub-period ($X^2=24.784$, d.f.=5, $p<0.05$), and tide level ($X^2=9.900$, d.f.=3, $p<0.05$).

All squeak call parameters (Table 4.16) analysed varied for calls produced during different times of the day (Table 4.17) but they did not vary enough to provide a strong correct call classification (only 34% correct classification). The same was observed for calls produced at different tide levels (Table 4.18), where only 43.8% of calls were correctly classified.

Table 4. 15. Chi-squared values for the number of calls produced per younger reintroduction-AL manatee per hour for each time sub-period and tide level.

Manatee group	**Time sub-period/tide level**	**X^2**	**Degrees of freedom**	**p-value**
Younger reintroduction males-AL	1am-5am	62.313	4	P<0.05
	5am-9am	62.727	4	P<0.05
	9am-1pm	118.574	4	P<0.05
	1pm-5pm	35.006	4	P<0.05
	5pm-9pm	83.541	4	P<0.05
	9pm-1am	43.541	4	P<0.05
	Flood	47.387	4	P<0.05
	Peak high	90.835	4	P<0.05
	Ebb	117.880	4	P<0.05
	Peak low	22.081	4	P<0.05

Table 4. 16. Average duration(ms), frequency of maximum energy (kHz), maximum frequency (kHz), minimum frequency (kHz) and bandwidth (kHz) for Squeak calls produced by younger reintroduction males throughout the day and tide regimes.

Manatee group	Time of day	Tide level	Duration (ms)	Frequency of maximum energy (kHz)	Maximum frequency (kHz)	minimum frequency (kHz)	Bandwidth (kHz)
Younger males	1am-5am	flood	246.505±58.473	3.432±0.434	4.010±0.542	3.267±0.583	0.742±0.499
	1am-5am	ebb	331.724±9.657	2.201±0.017	2.452±0.025	2.052±0.019	0.400±0.027
	1am-5am	peak high	317.230±11.079	2.182±0.019	2.461±0.023	2.052±0.014	0.409±0.026
	5am-9am	flood	353.762±15.509	2.186±0.017	2.593±0.027	1.880±0.016	0.713±0.039
	5am-9am	ebb	300.610±17.830	1.967±0.088	2.313±0.092	1.835±0.094	0.479±0.060
	5am-9am	peak high	302.690±18.469	2.640±0.193	3.009±0.220	2.330±0.180	0.678±0.137
	9am-1pm	flood	311.628±15.786	2.094±0.148	2.448±0.166	1.918±0.141	0.530±0.052
	9am-1pm	ebb	287.758±20.780	1.903±0.104	2.278±0.094	1.730±0.123	0.548±0.082
	9am-1pm	peak high	233.236±7.020	3.433±0.038	4.457±0.110	3.250±0.052	1.207±0.101
	1pm-5pm	flood	306.891±14.096	2.601±14.096	3.002±0.241	2.365±0.214	0.637±0.085
	1pm-5pm	peak high	305.237±11.618	2.423±0.151	2.541±0.120	2.145±0.100	0.396±0.039
	5pm-9pm	flood	320.225±15.582	2.210±0.322	2.505±0.328	2.044±0.310	0.461±0.039
	5pm-9pm	ebb	345.606±6.344	2.194±0.016	2.464±0.029	1.971±0.029	0.493±0.029
	5pm-9pm	peak high	213.870±19.655	1.701±0.233	1.971±0.237	1.507±0.290	0.464±0.077
	9pm-1am	flood	361.969±7.531	2.256±0.014	2.531±0.027	2.026±0.019	0.505±0.034
	9pm-1am	ebb	341.870±12.531	2.196±0.017	2.505±0.043	1.965±0.045	0.539±0.036
	9pm-1am	peak high	311.625±12.017	2.260±0.024	2.522±0.018	2.035±0.027	0.487±0.030

Table 4. 17. Contribution of squeak call parameters: Syllable duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz) to the differentiation of calls produced during different hours of the day by younger reintroduction males-AL (n=2).

Squeak call parameter	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Duration (ms)	0.874	4.222	5	147	0.001
Frequency of maximum energy	0.924	2.429	5	147	0.038
Maximum Frequency	0.914	2.760	5	147	0.021
Minimum Frequency	0.899	3.312	5	147	0.007

Table 4. 18. Contribution of squeak call parameters: Syllable duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and minimum frequency (kHz) to the differentiation of calls produced during different tide levels by younger reintroduction males - AL (n=2).

Squeak call parameter	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Duration (ms)	0.927	3.891	3	149	0.010
Frequency of maximum energy	0.805	12.019	3	149	<0.001
Maximum Frequency	0.747	16.849	3	149	<0.001
Minimum Frequency	0.824	10.573	3	149	<0.001

5.4 Discussion

Our study shows that different manatee groups present significantly different diel and tidal call patterns. We also found that all groups produced significantly more tonal, harmonic Squeak calls, compared to the other call types and that Squeak call structure differed according to time of day and tidal regimes.

We found differences in call rates between groups for the sub-periods from dawn until the evening (5am-9pm) and for all tide levels. However, no differences were found during the night sub-periods (9pm-5am). We observed differences within captive males, captive females and younger reintroduction males-AL for diel call rate patterns and within older reintroduction males-AL for tidal call rate patterns. Both the older and younger reintroduction males-AL presented the lowest call rates during the night and peak low tide. The captive males, captive females and reintroduction pair-PB presented peak call rates between 5am-9am. Similar patterns have been reported for sei whales, where significantly higher call rates were recorded during the day compared to the night (Baumgartner and Fratantoni, 2008) and for tropical and temperate odontocetes, where the probability of whistle production was significantly higher during the morning (Oswald et al., 2008). Additionally, blue whales also presented peak call rates immediately after sunrise and prior to sunset (Wiggins et al., 2005). It is important to note, however, that this pattern was only observed for the captive animals and the recently translocated manatee pair. Captive animals generally follow a regular routine and this time period, 5am-9am, also coincides with the morning feeding time at the ICMBio/CMA. A similar pattern was observed in captive bottlenose dolphin vocal behaviour, where whistle production rate increased with the arrival of husbandry staff (Probert et al., 2021) and in captive common dolphins, *Delphinus delphis*, who presented peak whistle rates between 8am-9am and 1pm-5pm which coincided with feeding periods (Moore & Ridgeway, 1996; Goold, 2006). The lowest call rates for the captive animals and the reintroduction pair-PB were observed during the hottest hours of the day (9am-1pm). This may be associated with the lack of shade in the captive centre and the restricted movement of the animals due to the relative sizes of the pools and manatees. Thus, it may not have been energetically beneficial for the manatees to vocalise during the day, due to the costs of thermoregulation. Low vocal production during the hottest periods of the day has also been observed for Southern right whales (Shabangu et al., 2020).

The reintroduction pair in Paraíba, presented the highest call rate between 1-5am, followed by 5am-9am, and during peak low tide. This may be explained by several factors: the reintroduction pair-PB were translocated from a captive setting (ICMBio/CMA) to a natural reintroduction environment, with no previous exposure to natural elements nor to the opposite sex. Thus, the vocal patterns of these translocated animals may reflect the patterns formed during their time in the ICMBio/CMA captive setting. Furthermore, it is very likely that the animals were experiencing high levels of stress, especially since they had no previous exposure to tidal regimes and, consequently, fluctuating depths, natural illumination, and the natural environment. This could partially explain the differences observed between the reintroduction pair-PB and the reintroduction males-AL, who were probably much more acclimated to their natural estuarine environment.

Curiously, we observed that differences in the call rates of the older and younger reintroduction males-AL, were more strongly influenced by tide regime and time of day, respectively. A similar finding was recorded for Indo-Pacific humpback whales in an estuarine environment, where the acoustic encounter rate differed in function of tidal cycle rather than diurnal cycle (Lin et al., 2013). Tide level was also found to significantly affect the vocalisation rate of dugongs in a feeding area (Tanaka et al., 2016). In our study, these findings may be explained by the position of the two pools, where the depth of the smaller older male pool changes more dramatically compared to the larger younger male pool. Thus, it is possible that the older males experience stronger impacts from tide fluctuations compared to the younger males. Here we used tide as an indirect measure of turbidity, since the concentration of dissolved substrates in the water column increases with decreasing tide level. The Tatuamunha estuary, in particular, has a relatively high silt content, especially in depths of less than 3m (Paiva, 2018). Therefore, we would expect that during low tide, i.e., depths of <1m, the silt content in the reintroduction pools would increase dramatically. However, it is also possible that these observed differences in call rate are due to age-related differences, as previously observed by Umeed et al. (2023).

Peak calling activity also varied between these two groups, where the call rate of the older males was highest between 5pm-9pm and between 9am-1pm for the younger males. Both groups, however, produced the lowest number of calls during the darkest period of the day and during peak low tide and the highest call rates during flood tides and peak high tides. Thus, our first hypothesis cannot be confirmed since we observed the opposite to what was expected. Barlow et al. (2018) found that the probability of detecting the feeding calls of North Pacific humpback whales, *Megaptera novaeangliae*, was significantly related to flood tides. Additionally, narwhal, *Monodon monoceros*, click detections were found to be more abundant during flood tides and at 9pm (Marcoux et al., 2017). Nonetheless, it is important to note that the reintroduction centre on the Riacho Tabatinga is exposed to free-living resident Antillean manatees that move up and down stream according to tide regime. As such, it is possible that the highest call rates during peak high tide and flood tide observed for these two groups, coincide with the contact of the study groups with free-living Antillean manatees (Umeed et al., 2023).

We found differences in the types of calls produced by all the study groups according to time of day and tide level. A study by Stienessen (1998) showed that Atlantic spotted dolphins, *Stenella frontalis*, produced a higher proportion of whistles during the day and a higher proportion of pulsed sounds during the night (Oswald et al., 2008). Additionally, common dolphins, *Delphinus delphis,* have been shown to present diurnal differences in vocal production, producing whistles, click trains and squeals during the day and predominantly click trains during the night (Evans & Awbury, 1988; Oswald et al., 2008). Deconto & Monteiro-

Filho (2015) found that Guiana dolphins produced significantly more whistles, burst pulses and low frequency noise during the night compared to the day. As expected, Squeaks were overwhelmingly the most frequently produced vocalisations throughout the day and during all tide regimes, accounting for between 58.6% to 93.0% of overall call budget, which is consistent with previous findings (Umeed et al., 2023, Chapter 3.1 of this thesis). This suggests that this call type suffers less propagation loss over distance compared to other call types. Notably, the vocal repertoire of the reintroduction pair-PB was comprised of 93.3% Squeak calls. Sousa-Lima et al. (2008) described the production of contact calls, which are structurally equivalent to Squeak calls here, of captive Antillean manatees in situations of isolation i.e., stressful conditions. The authors noted that the structure of each call was significantly different, suggesting that these contact calls contain information on individual identity (Sousa-Lima et al., 2008). Furthermore, Lilly (1963) notes that the distress whistles of dolphins are repeated many times until an appropriate response is prompted from a conspecific. Thus, the high stereotypy of the Squeak calls produced by the reintroduction pair-PB would indicate that they were experiencing high levels of stress and attempting to make contact with conspecifics. Therefore, our results further reinforce the notion that Squeak calls contain individual identity information and are likely used to maintain contact between conspecifics (Umeed et al., 2023, chapters 3.1 and 3.2 of this thesis).

Finally, we observed that the Squeak call structure of the captive females, reintroduction pair-PB and the older and younger reintroduction males-AL changes during different time periods and tide regimes, however, not to the extent that calls can be clearly differentiated based on time period/tide regime alone. Thus, we can partially confirm our hypothesis, since the calls of four out of the five manatee groups differed for 75-100% of the four structural variables included in the analyses, according to time period and tide regime. We would expect manatee calls to alter the structure of their calls to account for the changing abiotic conditions encountered in their habitats. Amorim et al. (2016) found that the structure of Amazon River dolphins changed significantly according to water turbidity and Deconto & Monteiro-Filho (2015) observed that Guiana dolphins produced lower frequency calls during the night. Here we found the opposite for captive animals, who produced Squeaks with lower maximum frequencies during the day (9am-9pm), compared to the night (9pm-9am). We also found structural differences in the calls produced by all three reintroduction groups. The older males produced calls with average higher maximum frequencies during flood tides and the lowest frequencies during the peak low tides, which is in accordance with the results of

Amorim et al. (2016). However, the younger males produced calls with the highest average frequencies at peak low tides and the lowest maximum frequencies at peak low tide. This finding further reinforces the idea of age-related differences in manatee vocal production. The frequencies of the reintroduction were relatively constant, suggesting that the animals were still adapting to their new natural environment. It is important to note that although we found differences for the four structural call parameters, we obtained low correct call classifications, which may be due to the small sample size of analysed calls for each group (n=10), especially since there was more than one individual in each group.

5.5 Conclusions

The results of our study indicate that the vocal pattern of captive manatees and younger reintroduction males is influenced by the time of day, and the vocal pattern of older reintroduction males is influence by tide level. We also found that manatees produce significantly greater amounts of tonal harmonic calls (Squeaks) compared to other call types, reinforcing the notion that these calls propagate well in captive and natural environments and that they potentially contain information about individual identity and are used to maintain contact with conspecifics. Squeak call structure was significantly different depending on the time of day and tide level, suggesting that manatees alter their vocal structure to adapt to abiotic fluctuations in their environment. To our knowledge, this is the first study on the diel and tidal vocal patterns of Antillean manatees in North-eastern Brazil and adds to our limited knowledge on the ecology and behaviour of this endangered species.

6 Article 4 - The effects of abiotic factors on Antillean manatees (*Trichechus manatus manatus*) call propagation through playback experiments.

6.1 Introduction

When underwater acoustic signals are transmitted, they experience a reduction in strength over distance due to several variables that affect sound propagation (Seghal et al. 2010). This reduction in acoustic intensity is known as propagation loss and is a consequence of three main factors, attenuation, reflection and geographic spreading (Seghal et al. 2010). The speed of sound propagation underwater depends on three main abiotic parameters: temperature, salinity and depth (Deconto et al., 2021). The higher the water temperature and salinity, the faster the speed of sound propagation (Au & Hastings, Deconto et al., 2021). The pH, on the other hand, affects sound absorption, which decreases a reduction in pH (Ilyina et al., 2009). However, variations in abiotic conditions may also indirectly impact marine mammal species. For example, a decrease in sound absorption in the oceans will result in both natural and anthropogenic sounds travelling further, which will, consequently, result in increased noise levels (Kloepper and Simmons 2014; Gazioglu et al. 2015). These alterations in the marine environment may potentially negatively affect marine mammal acoustic behaviour. However, these predictions have not been empirically tested in the ocean and are based on theoretical calculations (Kloepper and Simmons 2014).

The Antillean manatee, *Trichechus manatus manatus*, inhabits rivers, estuaries and shallow coastal waters ranging from North-eastern Brazil to the Caribbean (Garcia-Rodrigues et al., 1998; Costa, 2006) and is currently threatened with extinction in North-eastern Brazil. During the summer in some Brazilian coastal basins, estuaries are filled exclusively with sea water due to the absence of rainfall, resulting in elevated salinity in these areas (Dias, 2005; Favero et al., 2020). As such, manatees appears to experience both seasonal and diel abiotic fluctuations in their environments, for example, variations in water turbidity, temperature and salinity. Studies have shown that turbidity affects the acoustic behaviour of Amazon River dolphin, *Inia geoffrensis* (Amorim et al., 2016). Furthermore, depth affects the sound propagation of aquatic organisms (Forrest et al., 1993), and dissolved oxygen concentration affects the structure of Guiana dolphin, *Sotalia guianensis* whistles (Deconto et al., 2021).

Vocal communication plays a vital role in maintaining contact between conspecifics, especially between manatee mothers and calves (Sousa-Lima et al., 2008; Charrier et al., 2009;

Umeed et al., In Press). Antillean manatees produce vocalisations with fundamental frequencies ranging from 0.64 to 5.23 kHz (O'Shea & Poché, 2006; Umeed et al., 2018; Merchan et al., 2019; Umeed et al., 2023), reaching up to 150kHz (Ramos et al., 2020). Manatees produce calls that contain information on individual identity (Sousa-Lima et al., 2008; Umeed et al., 2023; Umeed et al., In Press). These calls present slight variations in frequency that are specific to individuals or mother-calf pairs (Umeed et al., In Press). The importance of recognising offspring is critical for mothers since parental care is an energetically costly investment (Trivers 1974; Charrier et al., 2009). Thus, we would expect manatees, especially females and calves, to have evolved adaptations to ensure the successful propagation of their calls within their habitats. Many marine mammal species present adaptations in their vocal behaviour in response to environmental variables. For examples, right whales, *Eubalaena glacialis* and *Eubalaena australis*, bottlenose dolphins, *Tursiops truncatus* and beluga whales, *Delphinapterus leucas,* produce lower call rates in high noise conditions (Parks & Clark, 2007; Luís et al., 2014; Lesage et al., 1999). Other studies have demonstrated the influence of seasonality and time of day on marine mammal call production (Baumgartner and Fratantoni, 2008; Oswald et al., 2008; Wiggins et al., 2005). However, there is a gap in knowledge about how call structures may be affected by abiotic water variables.

Here we aimed to investigate the underwater propagation of Antillean manatee calls over distance, and how pH, temperature and salinity affect the propagation of these calls. We predicted that with calls from females and calf presenting more stable call parameters compared to calls from males, i.e., calls from females and calves suffer the least propagation loss over distance and under different abiotic conditions. We also expected to find correlations between abiotic factors and call parameters over distance.

6.2 Material and Methods

6.2.1 Study area

We carried out our call propagation experiments in three natural pools at Serrambi Beach, Ipojuca – Pernambuco (Figure 4.2-1), between September 2020 and April 2021. This area was chosen because we have found manatee faeces on the beach in this area, and local fishermen have confirmed their presence in the area (Bezerra *pers. com.*). The pools were numbered Pool 1, Pool 2 and Pool 3 (Figure 5.1). The geographical coordinates were obtained using Google Earth. The length of each pool was measured from the two furthest points (i.e., maximum length), and the width was measured from the two closest points (i.e., minimum width), as well as the two furthest points (i.e., maximum width). The depth of each pool was measured at the minimum and maximum widths of each pool. Measurements were taken using a measuring tape. These pools were chosen due to their different forms, i.e., Pool 1 was long, thin and deep, Pool three was short and shallow, and Pool 2 was wide, long and deep (Table 5.1). The different pool dimensions result in varied ph, temperature and salinity measurements, allowing us to investigate their effects on manatee call propagation. The sediment type of pools 1 and 3 is sandy-calcareous, whereas the sediment of pool 2 had a higher proportion of quartz sand. The distance from the closest pool (pool 1) to the shore was approximately 145m.

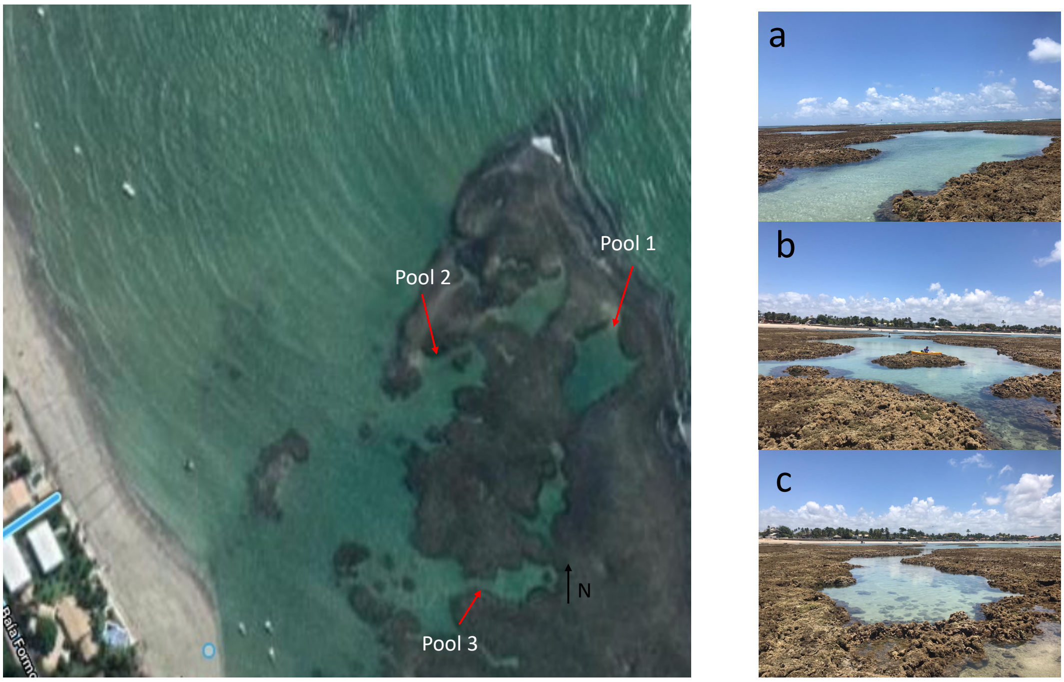


Figure 5. 1. Three natural pools in the reef of Serrambi Beach, Ipojuca – PE, where we conducted the call propagation experiments. Image sources: Google Earth Maps; and photos: Author).

Table 5. 1. Measurements and geographical coordinates of the natural pools included in this study. Coordinates were obtained using Google Earth.

Pool	Coordinates	Length(m)	Width(m)		Depth(m)	
			Minimum	Maximum	Minimum	Maximum
1	8°33’32”S 35°00’19”W	41	3.40	17.30	0.50	1.20
2	8°33’31”S 35°00’21”W	49	4.70	18	1.30	1.58

3	08°33’35”S 035°00’20”W	16	2	7	0.50	0.50

6.2.2 *Abiotic variables*

The abiotic variables considered in this study were surface temperature, surface pH and surface salinity. Each trial was carried out under differing abiotic conditions (see Table 5.2 for details on the abiotic measurements for each trial in each pool). We calculated the average pH, temperature and salinity values from three measurements taken at the start, end and middle of each pool during each trial. Temperature and pH were measured using DANOPLUS Water quality tester kit 6 in 1, and water samples were collected to measure salinity posteriorly in the laboratory using a refractometer model VX100SG.

Table 5. 2. Average pH, temperature and salinity values for each trial in each pool.

	Pool 1				**Pool 2**				**Pool 3**			
Trial	pH	Temperature (°C)	Salinity (ppt)	Tide level (m)	pH	Temperature (°C)	Salinity (ppt)	Tide level (m)	pH	Temperature (°C)	Salinity (ppt)	Tide level (m)
1	8.28	26.83	37.67	0	8.26	28.60	37.67	0	8.35	27.60	37.67	0
2	8.27	26.57	39.00	0	8.25	27.70	40.00	0	8.37	28.80	40.00	0
3	8.24	27.03	39.33	0	8.02	29.14	38.00	0.1	8.38	29.57	39.00	0
4	8.04	29.83	38.33	0	8.02	27.67	37.67	0	8.2	27.20	39.33	0
5	7.78	28.70	38.67	0.3	8.03	28.87	38.00	0	7.96	27.40	37.33	0.1
6	8.05	28.67	38.33	0.0	8.05	28.83	39.00	0.1	8.11	27.73	39.33	0

6.2.3 Call propagation experiments

We used the vocalisations of five manatee call groups, previously recorded by RU (Umeed et al., 2023; unpublished data): calls recorded in a pool containing six captive females, calls recorded in a pool containing four captive males, calls recorded in a reintroduction pool containing two older males, calls recorded in a reintroduction pool containing two younger males and, calls recorded in a pool containing one isolated captive calf. The captive animals (males, females and calf) were recorded at the Instituto Chico Mendes de Conservação da Biodiversidade/ Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos (ICMBio/CMA), Ilha de Itamaracá – PE and the reintroduction individuals were recorded in two reintroduction pools in the Área de Proteção Ambiental (APA) Costa dos Corais, Porto de Pedras – AL (see Chapter 3.1 for details on individual manatees).

All the vocalisations used for the propagation playback experiments were recorded with a SM4 Wildlife Acoustics recorder (linear frequency response 20Hz-48kHz) attached to a SM3 hydrophonex hydrophone. We used 10 samples from each call group. Thus, a total of 50 manatee vocalisations were used for the playback experiments, i.e., 10 Squeak vocalisations from each animal group. Squeak vocalisations were selected as they are produced by all the study animals and have been suggested as containing information on individual identity (Sousa-Lima et al., 2008; Umeed et al., 2023; Umeed et al., In Press).

We performed six trials in each natural pool. Each vocalisation was separated by a one-second recording of water, recorded in the captive pools at the ICMBio/CMA. The call playbacks were played underwater using a Lubell's labs LL916 underwater speaker with an AC203E Circuit Master (linear frequency response 200Hz-23kHz +/-10dB) connected to an iPhone 7S. The playback vocalisations were recorded using two SQ26-H1 Cetacean Research hydrophones with Zoom H1 recorders (linear frequency response: 20Hz- 20kHz). Two researchers recorded the playbacks at alternating distances, i.e., researcher 1 recorded at 2m, while researcher 2 simultaneously recorded at 4m. One researcher held the speaker mid-way between the surface and the seafloor. The sounds were played back at distances of 2m, 4m, 8m, 16m and, where possible, 32m. These distances were marked on a rope which was placed at the surface of the pool to enable the researchers to stand at the correct distance for each repetition. We only conducted the experiments at low tide (up to 0.3), when the natural pool borders were fully exposed (Figure 5.1).

6.2.4 Call analysis and Statistical analyses

The playback recordings were analysed using Kaleidoscope Pro 5, Wildlife Acoustics software (512 samples for FFT, time resolution of 5.33ms, 50% overlap). We measured call duration (ms) and the bandwidth (kHz) and relative amplitude (dB) of the fundamental harmonic, where possible, for each vocalisation. These structural variables were selected as previous studies have shown the influence of abiotic variables on these parameters (Amorim et al., Deconto et al., 2021). When we could not identify the fundamental harmonic, the parameters of the first visible harmonic were measured.

We calculated the average call parameter values for each study group during each trial per pool in order to test whether there were differences in call parameters over distance (m). We used this data to perform Kruskall-Wallis tests. We also calculated the average call parameter values for each at 8m. We selected the distance of 8m since this was the maximum distance at which the fundamental frequencies of all calls could still be observed. We ran a Spearman's correlation between abiotic variables and each call parameter for each call group. We also calculated the average of each call parameter during each trial in each pool at 8m, in order to indirectly test for the effect of pool dimension on sound propagation. This was also achieved using Kruskal Wallis analyses. All analyses were performed on Prism 9 software, and significance was recorded as $p<0.05$.

6.3 Results

6.3.1 Call parameter propagation over distance

We found a difference in call duration (ms) over distance (m) for captive female calls (Table 4.2-3 Figure 5.2) and in bandwidth (kHz) for captive females and older males calls over distance (m) (Table 5.4 Figure 5.33). Additionally, the average relative amplitude (dB) over distance (m) was significantly different for all the study groups (Table 5.5, Figure 5.4).

Table 5. 3. Results of Kruskal Wallis tests performed on each call group for average duration (ms) in relation to distance (m). * Indicates significant values.

Call group	**Kruskal Wallis Statistic**	**d.f.**	**p-value**
Captive female	15.78	5	0.0033*
Captive males	6.559	5	0.161
Older males	4.361	5	0. 3593
Younger males	5.996	5	0.2017
Calf	3.234	5	0.5194

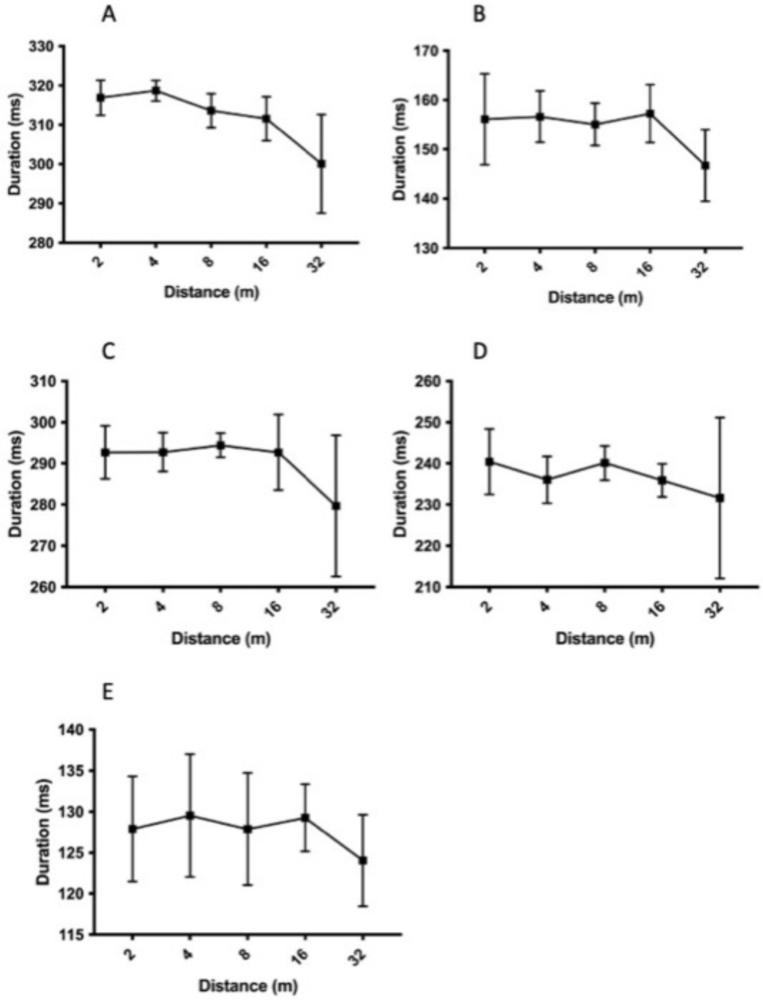


Figure 5. 2. The average values of the call parameter "duration" (ms) over distance (m) for each study call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males, E - calls from a calf

Table 5. 4. Results of Kruskal Wallis tests performed for average bandwidth (kHz) over distance for each call group. * Indicates significant values.

Call group	**Kruskal Wallis Statistic**	**d.f.**	**p-value**
Captive females	14.23	5	0.0066*
Captive males	3.54	5	0.4719
Older males	10.19	5	0.0374*
Younger males	6.478	5	0.1662
Calf	5.508	5	0.2391

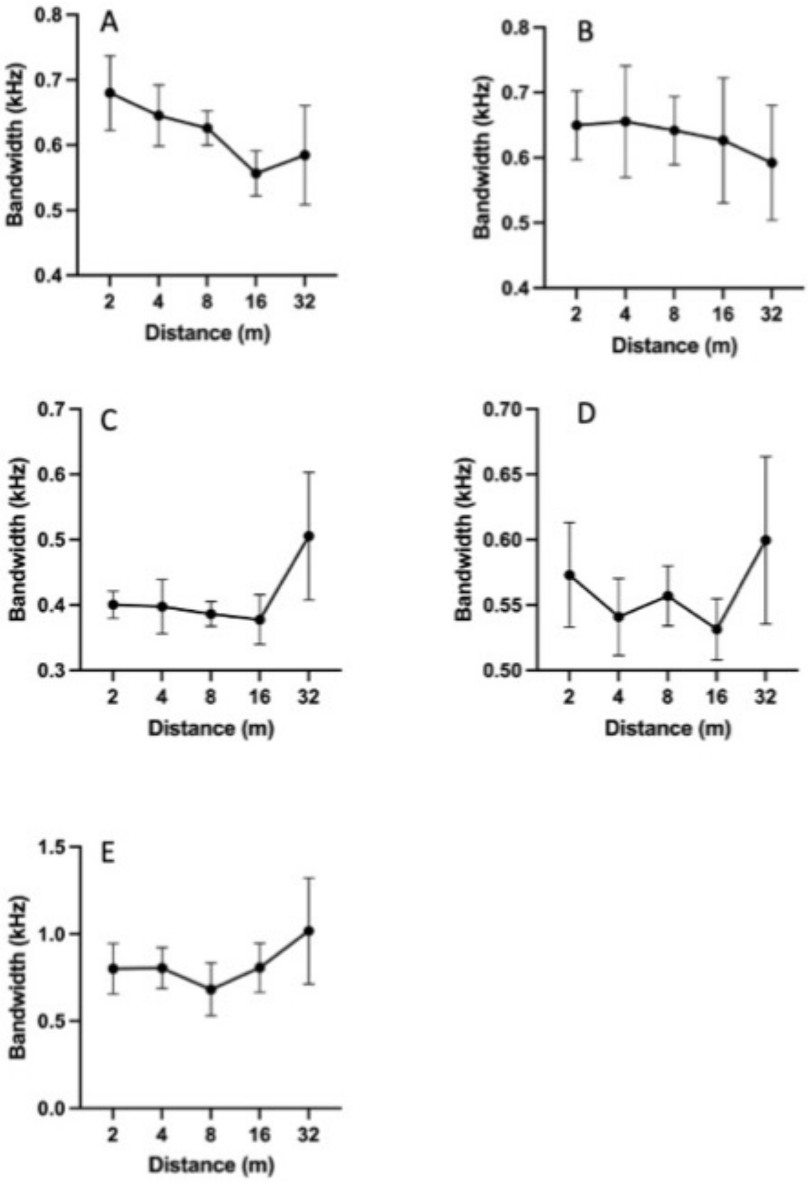


Figure 5. 3. The average values of the call parameter "bandwidth (kHz)" over distance (m) for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males, E — calls from a calf.

Table 5. 5. Results of Kruskal Wallis tests performed on each call group for average relative amplitude (dB) over distance (m). Results were significant for all study groups.

Call group	**Kruskal Wallis Statistic**	**d.f.**	**p-value**
Captive females	25.65	5	<0.0001
Captive males	24.69	5	<0.0001
Older males	24.73	5	<0.0001
Younger males	13.72	5	0.0083

Calf	17.24	5	0.0017

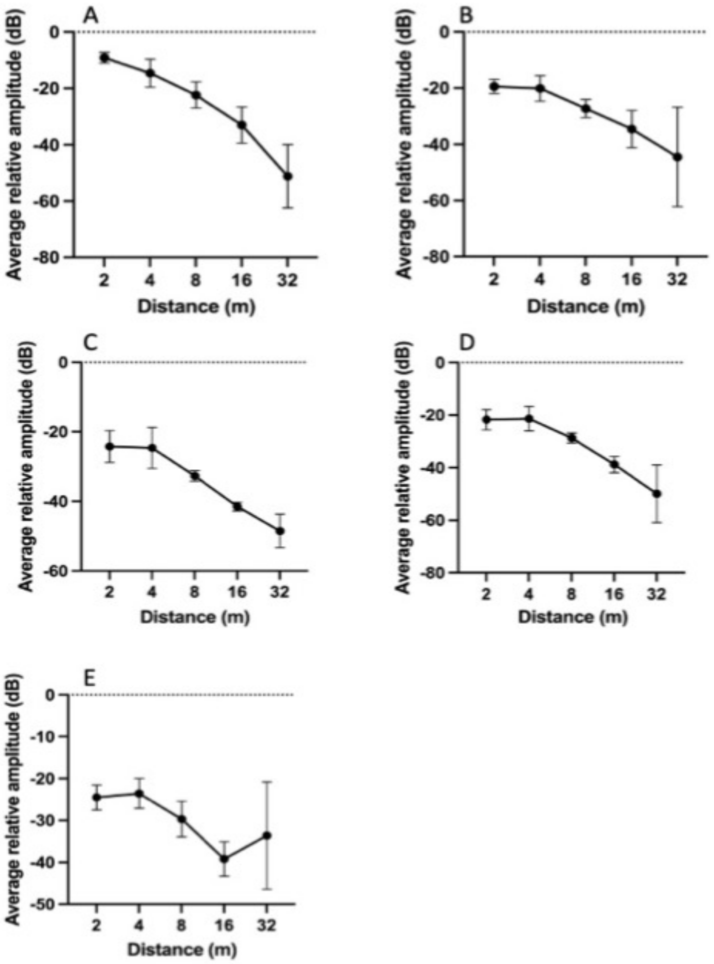


Figure 5. 4. The average values of the call parameter “amplitude (dB)” over distance (m) for each call group: A – calls from captive females, B – calls from captive males, C – calls from

older reintroduction males, D – calls from younger reintroduction males, E – calls from a calf. Dotted lines indicate zero values on the Y axis.

6.3.2 Correlations between call parameter propagation and abiotic variables (pH, temperature and salinity)

We found positive correlations between average relative amplitude and pH for calls produced by captive females, between average bandwidth and temperature for calls produced by captive males and between average bandwidth and temperature for calls produced by the calf (Table 5.6). We found a negative correlation for pH and average duration for calls produced by older reintroduction males (Table 5.6) (Figures 5.5 to 5.13).

Table 5. 6. Correlation between call parameters and the abiotic variables pH, temperature (°C) and salinity (ppt) at 8m.* Indicates significant values.

Call group	**Call variable**	**Abiotic variable**	**Spearman's correlation (r)**	**p-value**
Captive females	Duration (ms)	pH	0.1891	0.4522
		Temperature	0.09598	0.7048
		Salinity	0.3798	0.1201
	Bandwidth (kHz)	pH	0.1953	0.4373
		Temperature	0.07534	0.7664
		Salinity	0.2441	0.3289
	Relative amplitude (dB)	pH	0.5878	0.0103*
		Temperature	-0.3263	0.1864
		Salinity	0.1336	0.5971
Captive males	Duration (ms)	pH	0.184	0.4649
		Temperature	-0.3106	0.2096
		Salinity	0.1002	0.6925
	Bandwidth (kHz)	pH	0.03514	0.8899
		Temperature	1	<0.0001*
		Salinity	0.2441	0.3289
	Relative amplitude (dB)	pH	0.6880	0.0016*
		Temperature	-0.2478	0.3215
		Salinity	0.1628	0.5187
Older reintroduction males	Duration (ms)	pH	-0.5426	0.0200*
		Temperature	-0.3230	0.1911
		Salinity	-0.2577	0.3019

	Bandwidth (kHz)	pH	-0.2357	0.3465
		Temperature	0.4469	0.0630
		Salinity	0.04382	0.8629
	Relative amplitude (dB)	pH	0.1705	0.4989
		Temperature	0.1693	0.5018
		Salinity	0.2681	0.282
Younger reintroduction males	Duration (ms)	pH	-0.2212	0.3777
		Temperature	0.07327	0.7726
		Salinity	-0.03547	0.8889
	Bandwidth (kHz)	pH	-0.3152	0.2026
		Temperature	0.1785	0.4784
		Salinity	0.0793	0.7545
	Relative amplitude (dB)	pH	0.4339	0.0720
		Temperature	-0.03304	0.8964
		Salinity	0.1231	0.6265
Calf	Duration (ms)	pH	0.4184	0.0955
		Temperature	-0.1691	0.5152
		Salinity	0.134	0.6056
	Bandwidth (kHz)	pH	-0.08344	>0.9999
		Temperature	0.5906	0.0142*
		Salinity	0.09926	0.7029
	Relative amplitude (dB)	pH	0.3691	0.1448
		Temperature	-0.07725	0.7674
		Salinity	0.09926	0.7029

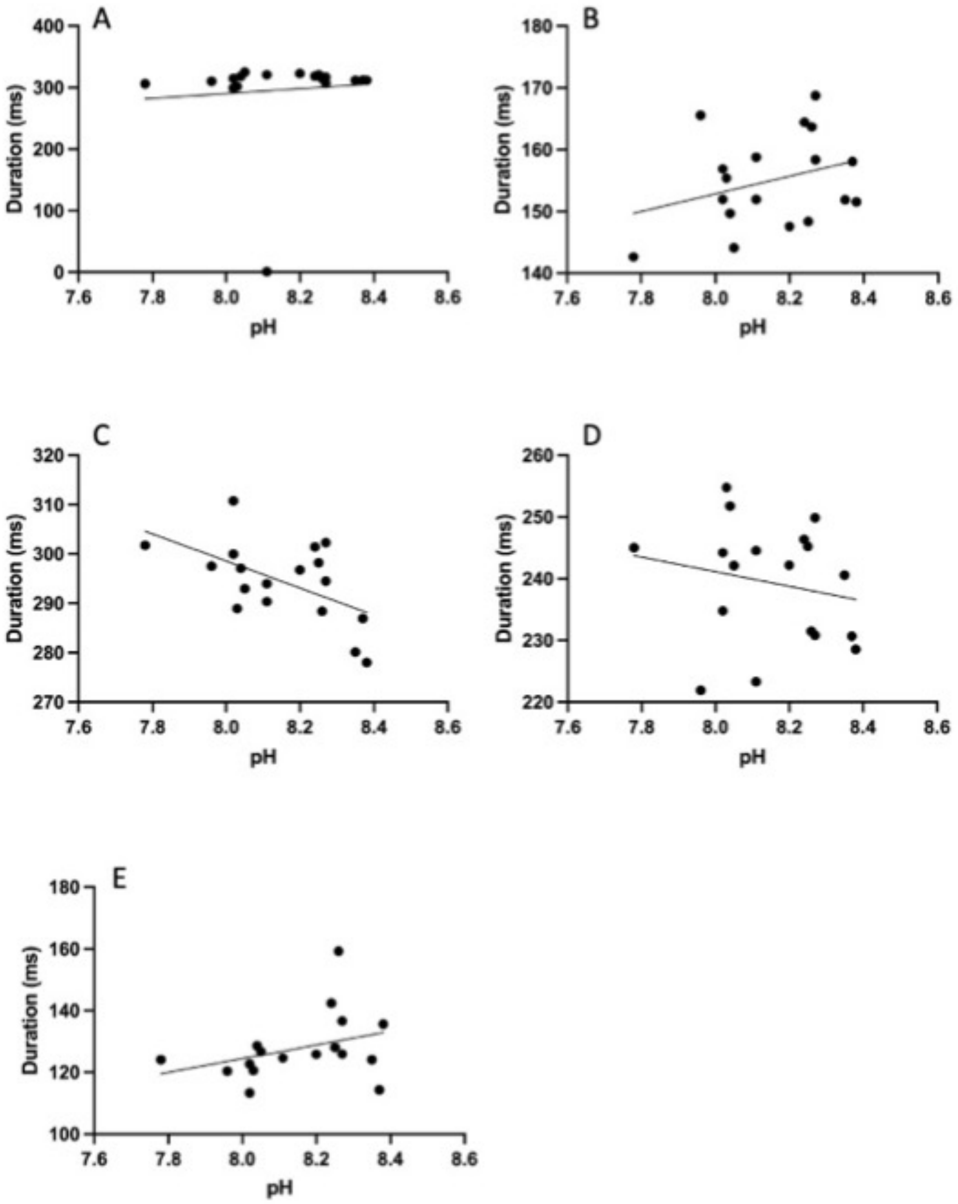


Figure 5. 5. The average values of the call parameter “duration (ms)” under different pH conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males and E – calls from a calf. A negative correlation was found for the older reintroduction males (C) ($p<0.05$).

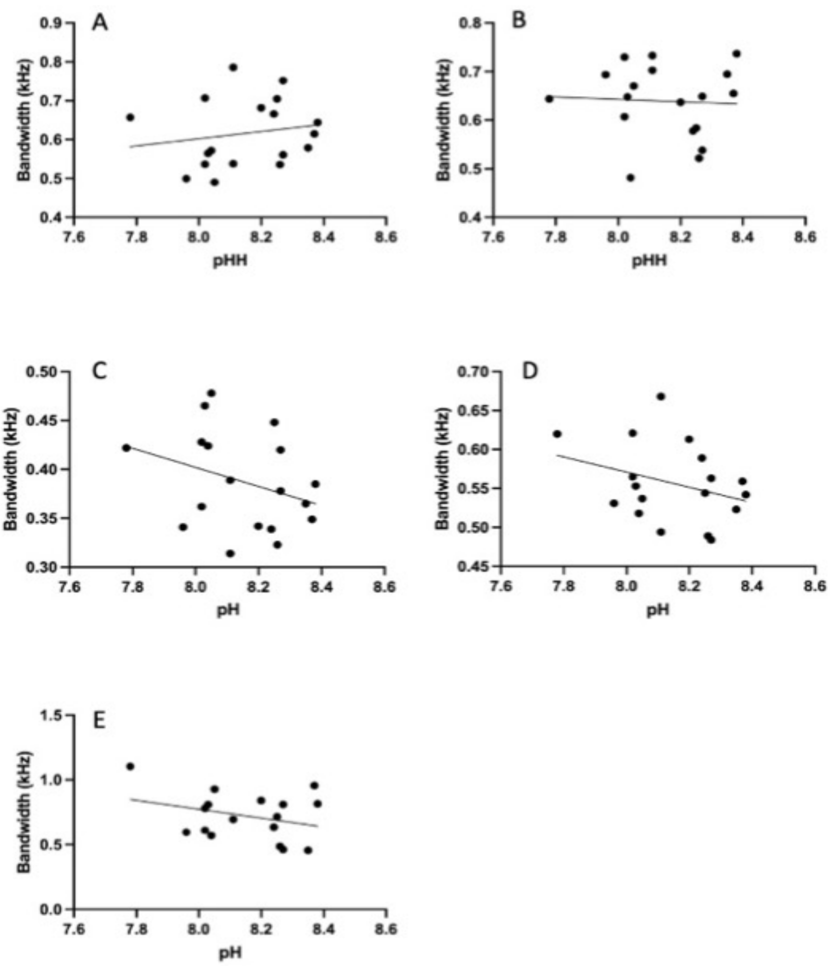


Figure 5. 6. The average values of the call parameter “bandwidth (kHz)” under different pH conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males and E – calls from a calf. No correlations were found between bandwidth and pH.

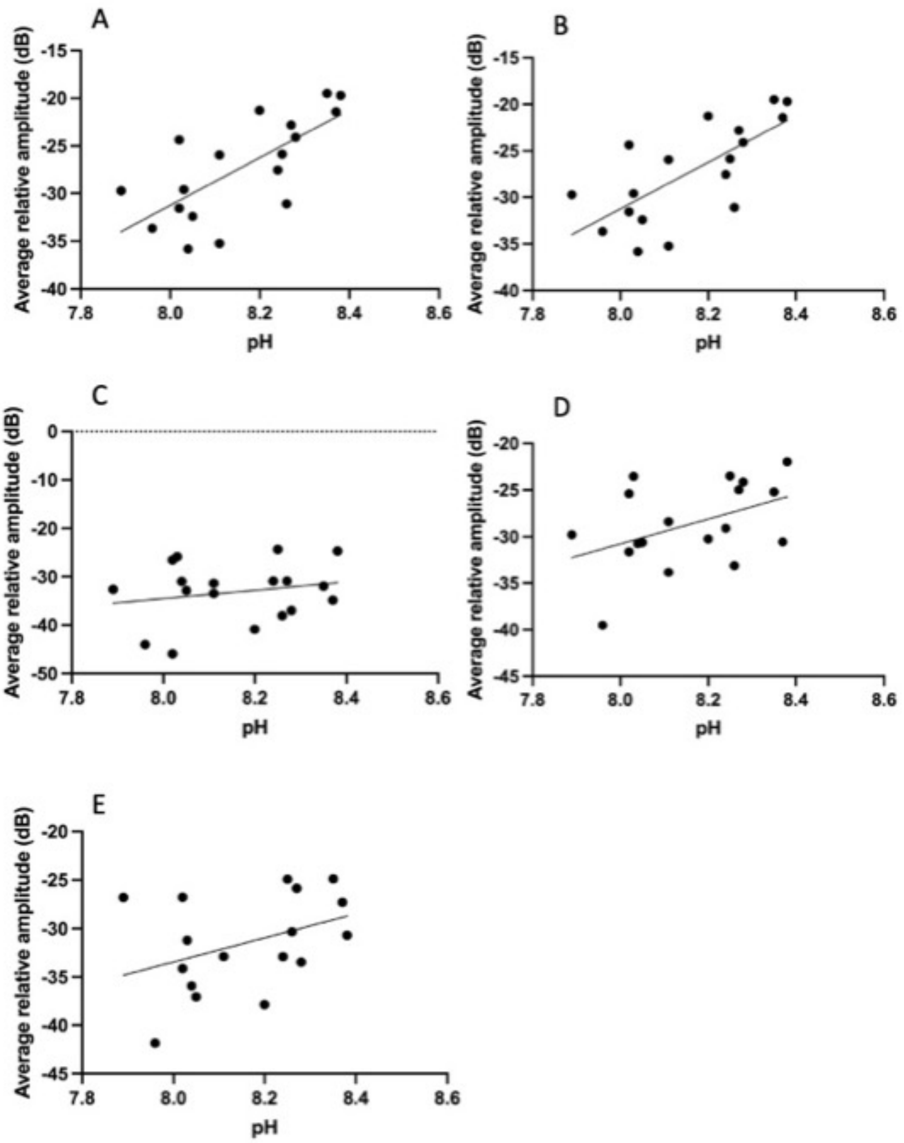


Figure 5. 7. The average values of the call parameter “amplitude (dB)” under different pH conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males and E – calls from a calf. Positive correlations were found for captive females and males (A-B) ($p<0.05$). Dotted line indicates the zero value on the Y axis.

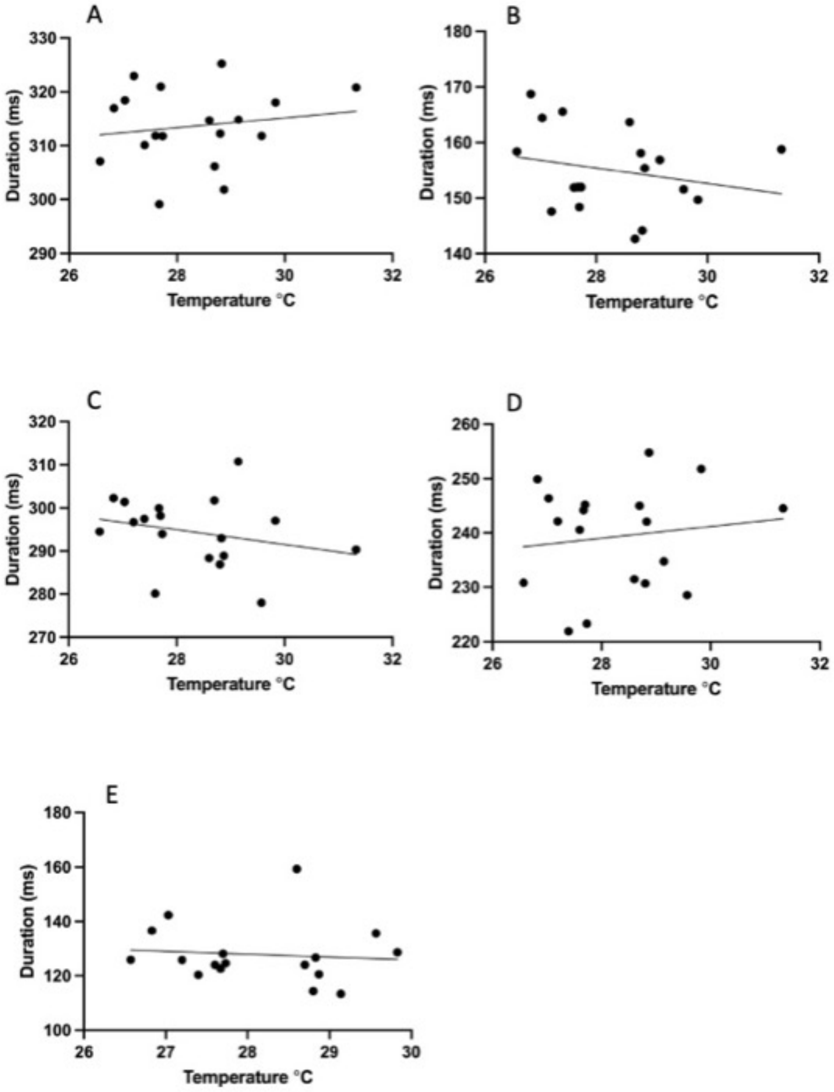


Figure 5. 8. The average values of the call parameter “duration (ms)” under different temperature conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger

reintroduction males and E – calls from a calf. No correlations were found between duration and temperature.

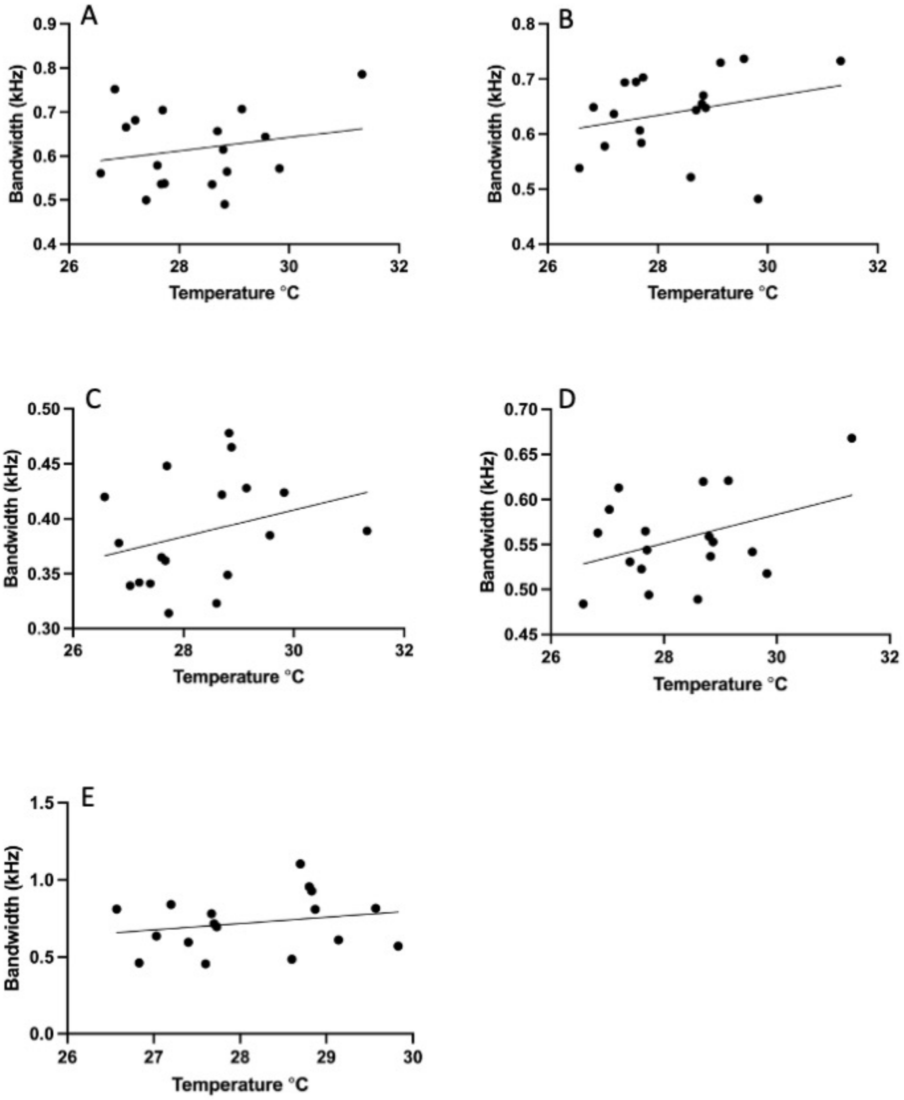


Figure 5. 9. The average values of the call parameter "bandwidth (kHz)" under different temperature conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger males and E – calls from the calf. A positive correlation was found for calls produced by captive males and the calf (B, E) ($p<0.05$).

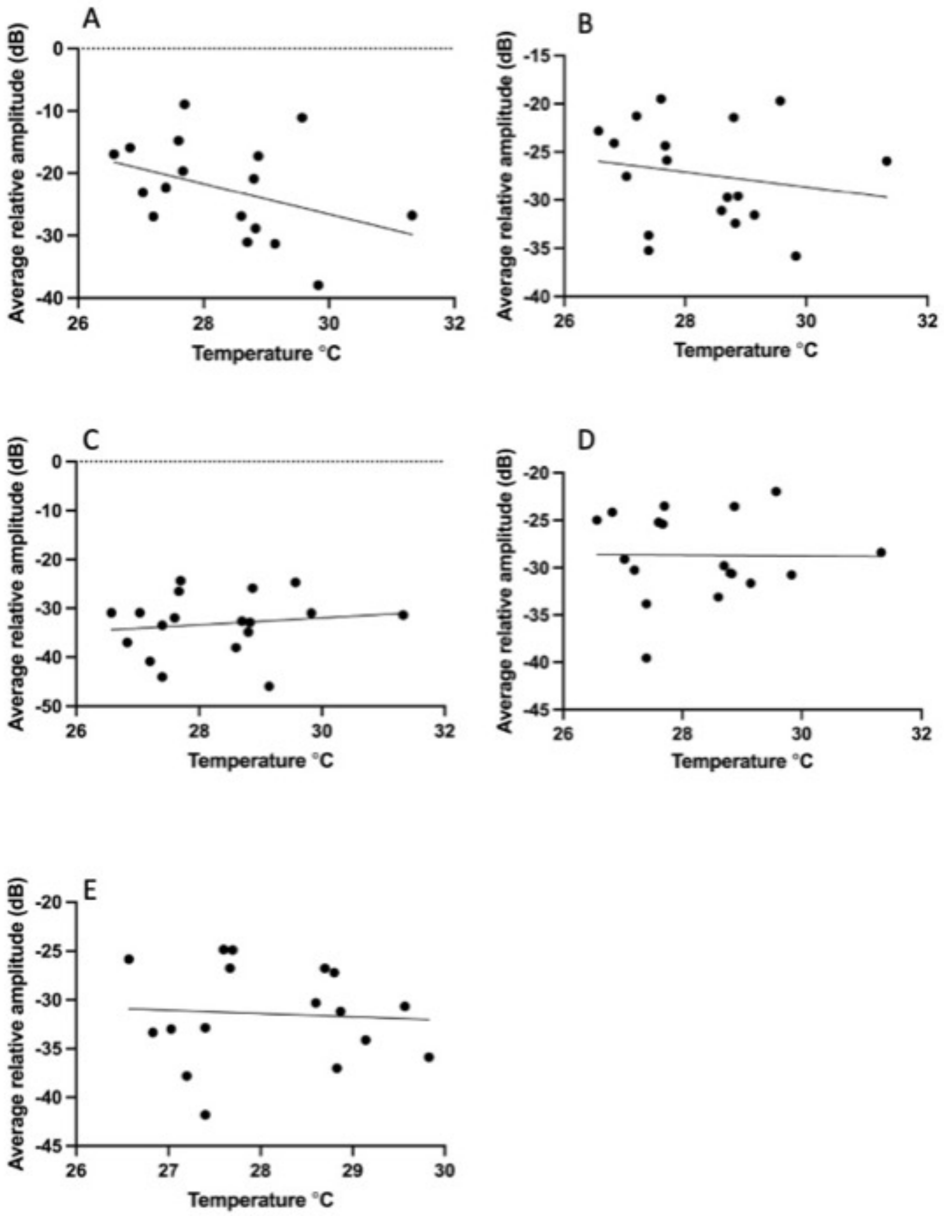


Figure 5. 10. The average values of the call parameter "relative amplitude (dB)" under different temperature conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males and E – calls from a calf. No correlations were found between average relative amplitude and temperature. Dotted line indicates the zero value on the Y axis.

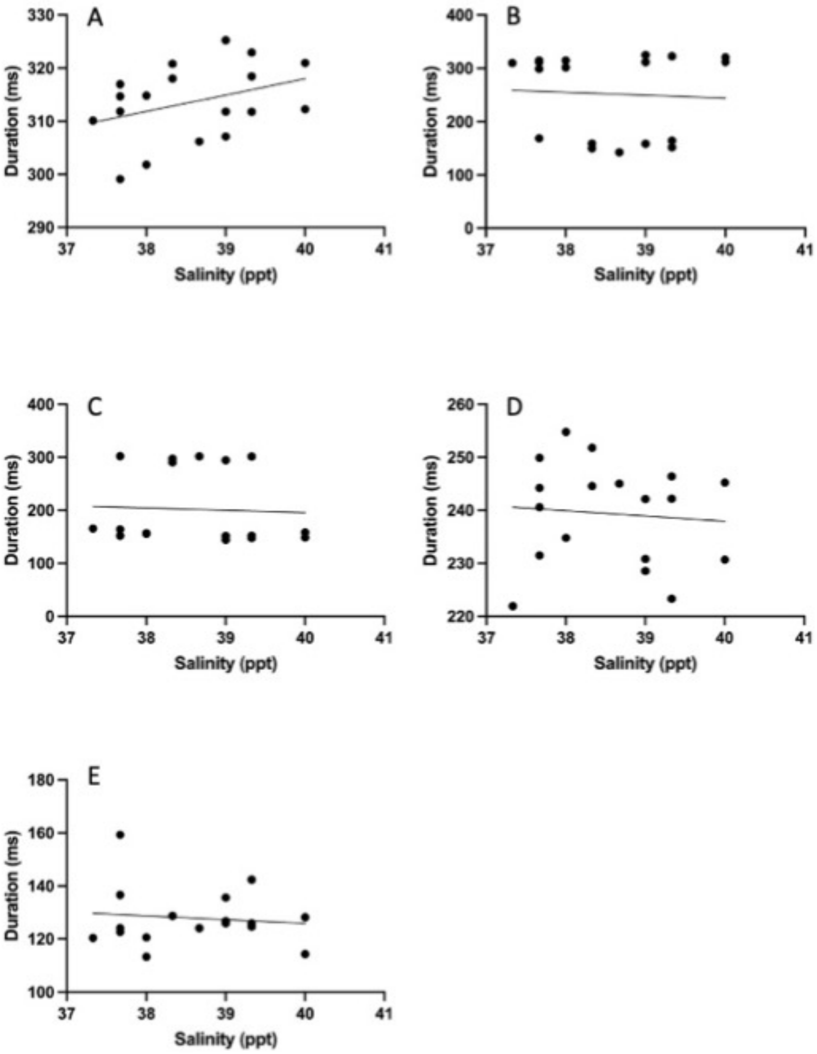


Figure 5. 11. The average values of the call parameter “duration (ms)” under different salinity conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males a and E – calls from a calf. No correlations were found between average relative duration and salinity.

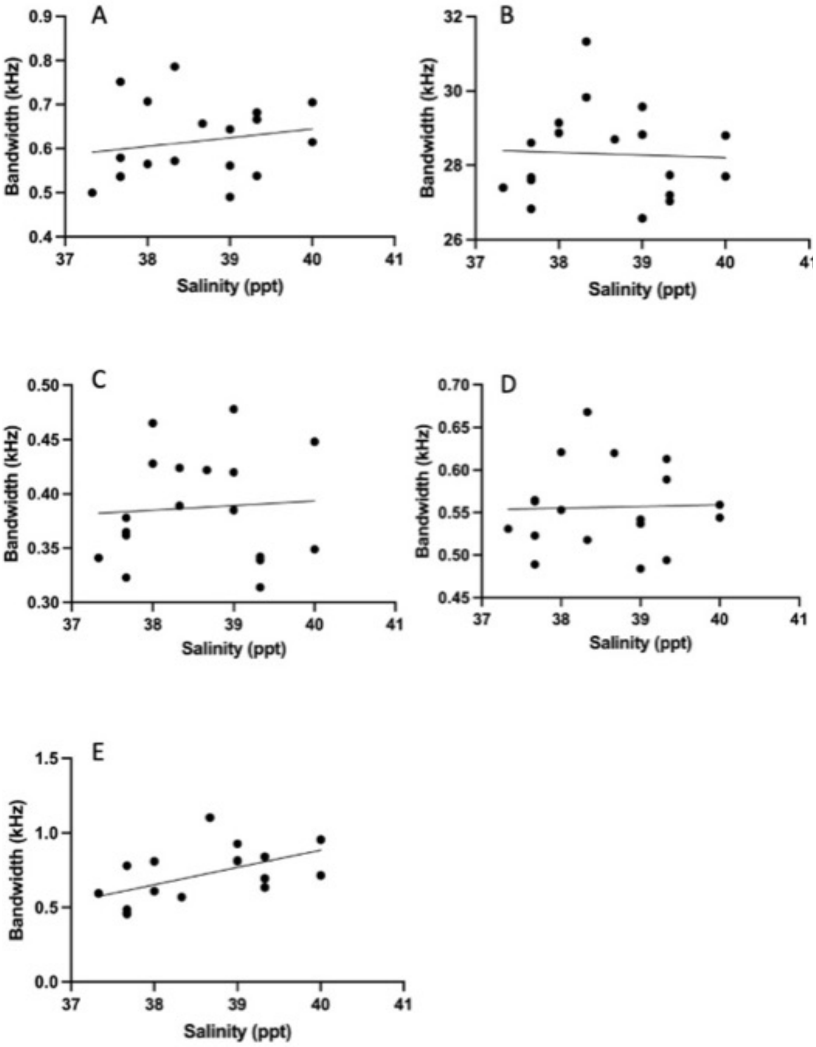


Figure 5. 12. The average values of the call parameter “bandwidth (kHz)” under different salinity conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males and E – calls from a calf. No correlations were found between bandwidth and salinity.

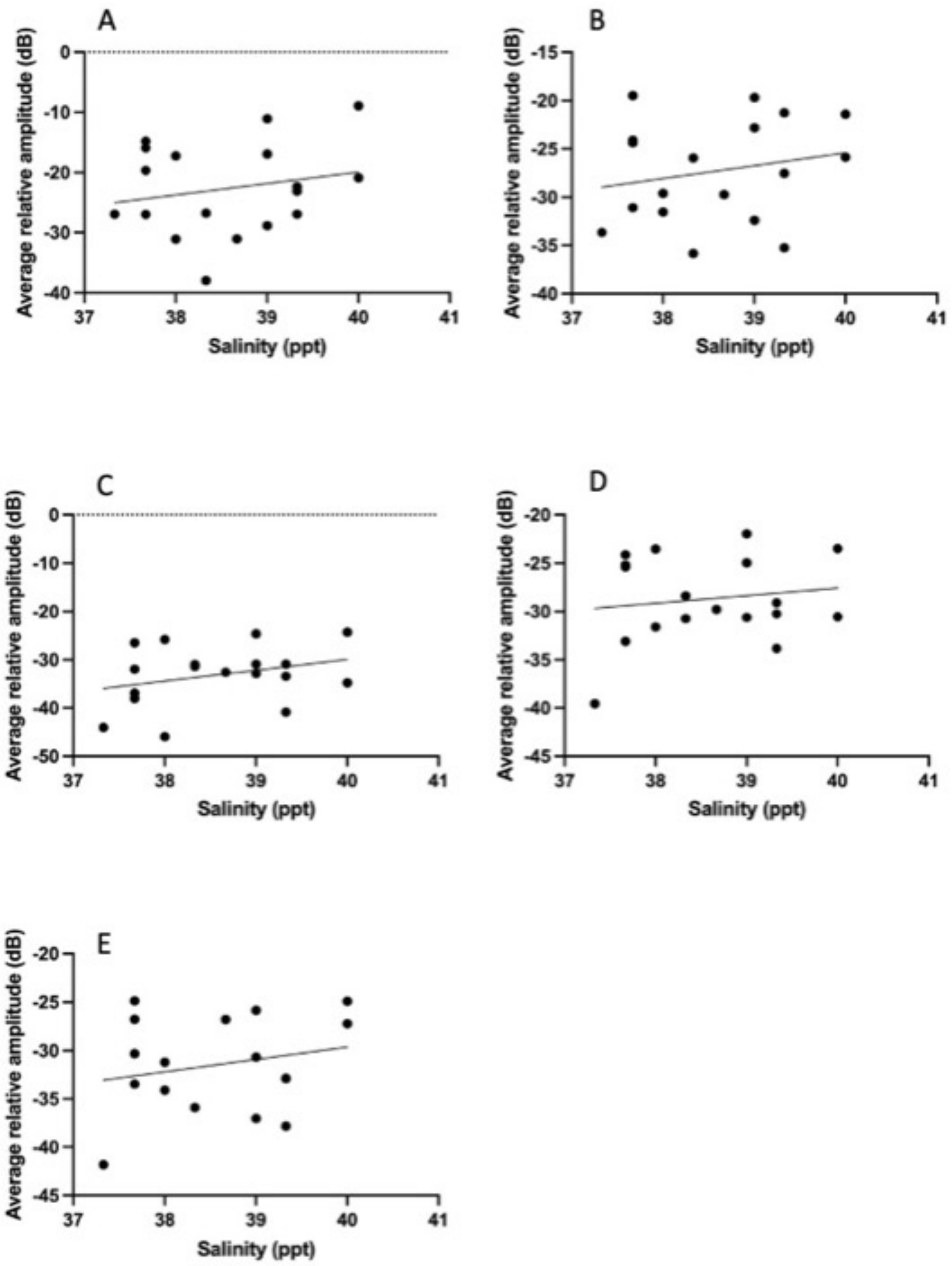


Figure 5. 13. The average values of the call parameter “relative amplitude (dB)” under different salinity conditions for each call group: A – calls from captive females, B – calls from captive males, C – calls from older reintroduction males, D – calls from younger reintroduction males and E – calls from a calf. No correlations were found between average relative amplitude and salinity.

6.3.3 The propagation of call parameters according to natural pool size

We only found differences for the duration (ms) of younger male calls at 8m (Table 5.7, Figures 5.14), suggesting that pool dimensions did not significantly affect sound propagation over distance.

Table 5. 7. Average values for call parameters of calls produced by all study groups at 8m. A difference was only found for younger reintroduction male call duration ($p<0.05$).

Call group	**Call variable**	**Kruskal Wallis Statistic**	**d.f.**	**p-value**
Captive females	Duration (ms)	0.2456	2	0.8968
	Bandwidth (kHz)	2.14	2	0.3596
	Relative amplitude (dB)	1.205	2	0.5728
Captive males	Duration (ms)	0.924	2	0.6522
	Bandwidth (kHz)	3.801	2	0.156
	Relative amplitude (dB)	1.906	2	0.4075
Older reintroduction males	Duration (ms)	4.363	2	0.1101
	Bandwidth (kHz)	5.135	2	0.0725
	Relative amplitude (dB)	1.275	2	0.5570
Younger reintroduction males	Duration (ms)	7.053	2	0.0220*
	Bandwidth (kHz)	0.8538	2	0.6828
	Relative amplitude (dB)	0.667	2	0.7414
Calf	Duration (ms)	2.889	2	0.2475
	Bandwidth (kHz)	0.1294	2	0.9435
	Relative amplitude (dB)	0.2092	2	0.9075

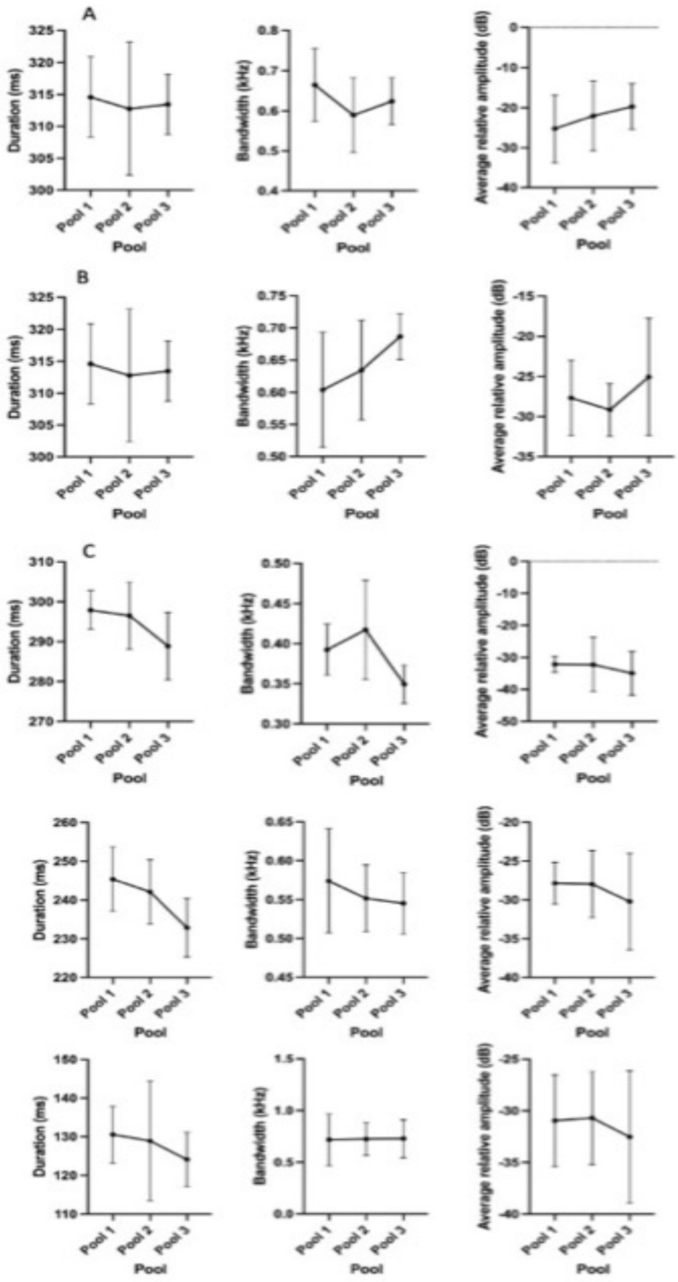


Figure 5. 14.The average values of each call parameters in each pool. A difference was only found for the duration of younger reintroduction male calls.

6.4 Discussion

Here we found that the average duration and bandwidth of captive female calls and the bandwidth of older reintroduction male calls differed over distance. We also found that average relative amplitude differed significantly over distance for all study groups. We found very few correlations between abiotic variables and call parameters and the duration of only one group call (i.e., calls from younger reintroduction males) was significantly different between pools, suggesting that pool dimensions did not significantly affect sound propagation for the analysed call parameters.

We did not observe differences over distance for most of the call variables and call groups. In a study on Guiana dolphins, Deconto et al. (2021) found that the duration, maximum and minimum frequencies of dolphin whistles did not vary significantly over distance. Our results reinforce this finding, where bandwidth indirectly represents maximum and minimum frequencies. The parameters of the calls produced by captive females presented more differences over distance compared to the other study groups. This was contrary to our expectations, where we expected female calls to be the most stable over distance. Communication between mothers and calves is vital for calf survival (Hartman 1979; Sousa-Lima et al., 2008; Miksis-Olds et al. 2009). Studies have demonstrated that mother and calf manatee pairs produce calls that are significantly structurally similar and are significantly structurally different to other mother calf pair calls (Umeed et al. In Press). Therefore, due to the importance of call transmission to offspring survival, we would expect female calls to propagate successfully in varying environmental conditions. However, our study has a limitation since we only included calls from captive females that have spent many years or even their entire lives in a captive setting. The structure of the female calls could have been initially affected by the captive setting. Thus, future studies should investigate the propagation of calls from free-living female manatees to further test this prediction.

Here we found that calls parameters degraded at a greater rate between 16m and 32m. Playback studies with Australian sea lions demonstrated that calls were substantially more degraded at 32m compared to shorter distances (Charrier et al., 2009; Pitcher et al., 2012). Furthermore, the amplitude and frequency modulations of harbour seal, *Phoca vitulina*, calls were found to degrade differently with increasing distance, where frequency spectrum was the least degraded acoustic feature and amplitude was the most degraded feature (Sauvé et al., 2015). The frequency parameters of Atlantic walrus, *Odobenus rosmarus rosmarus*, barks were also found to be highly resistant to degradation during propagation in playback experiments, suggesting that these frequency parameters may be the most important acoustic feature for individual identification (Charrier et al., 2009). Notably, we observed that average relative amplitude degraded more compared to frequency and duration parameters. Thus, our findings appear to agree with previous studies. Research on Antillean manatees has shown that frequency and duration parameters are key to defining individual manatee vocalisations (Sousa-Lima et al., 2008; Umeed et al., 2018; Umeed et al., 2022; Umeed et al., 2023). Thus, the relative stability of the Antillean manatee call frequencies included in our study, over distance and under different abiotic conditions, suggest that frequency parameters play an important role in individual identification. In estuaries, variability in sound absorption for temperature and salinity are particularly pronounced above 2kHz, and pH-dependent changes in absorption occur for frequencies below 3kHz (Miller et al., 2014). Studies have shown that the average maximum fundamental frequency of manatee calls falls between 0.64 to 5.23 kHz (O'Shea & Poché, 2006; Umeed et al., 2018; Merchan et al., 2019; Umeed et al., 2022), further suggesting that manatee calls are structurally well-adapted to environmental changes in their habitat.

We found correlations between pH and average relative amplitude for captive females calls and pH and call duration for older reintroduction male calls. We also found correlations between temperature and bandwidth for captive males and the calf. We did not find any correlations between salinity and the call parameters. This suggests that salinity does not have much influence on the underwater propagation of the study manatee calls, or at least less influence compared to pH and temperature. Notably, salinity was found to have the least effect on the propagation of sea trout, *Cynoscion nebulosus,* calls in three different environments (Briggs & Erisman, 2021). Furthermore, a study on Antillean manatees in Belize found that manatees visited sites with higher salinities significantly more than sites with lower salinity (Wistos, 2022). This may explain the lack of effect of salinity on call propagation observed here, since manatees may indeed prefer environments with greater salinities, thereby suggesting that this variable does not negatively affect their ability to communicate vocally successfully.

The abiotic variables, pH and temperature, influenced different call parameters. Notably, these variables only influenced the call parameters of the manatees that have been kept or continue to live in captive conditions (captive females, captive males and older reintroduction males). It is important to note that at the time of the study, the older reintroduction males had spent upwards of 20 years in the captive ICMBio/CMA centre, before being relocated to the reintroduction centre. Thus, it is possible that the calls produced by manatees in captivity, or those who have spent extensive periods of time in captivity, are more susceptible to propagation loss under different environmental conditions. This is expected since at the ICMBio/CMA, the abiotic conditions are kept relatively constant (Umeed, unpublished data), compared to the younger reintroduction manatees, who have spent the majority of their lives in a natural environment. Furthermore, Forrest (1993) suggests that short-range signals produced in shallow water, likely have frequencies below the frequency cut-off, and therefore, calls will not propagate far. The results of our study suggest that the calls produced by the captive manatees and older reintroduction manatees may be more suited to short-range communication, which would explain the higher levels of call degradation under different abiotic conditions. This can be justified by the close proximity of individual manatees to one another in the ICMBio/CMA oceanariums and the smaller size of the older reintroduction manatee pool compared to the younger reintroduction manatee pool, thereby removing the necessity of long-range communication. Thus, it is possible that the younger male calls have properties that promote propagation over distance (Forrest, 1993).

Since we found very few correlations between abiotic variables and call parameters, we can assume that other factors may influence Antillean manatee sound propagation more strongly (Deconto et al., 2021). Turbidity, for example, likely plays a very important role in sound propagation, which was not accounted for during this study. In a study on Amazon River dolphins, Amorim et al. (2016) found that frequency parameters, including bandwidth, were lower in more turbid water. The authors posit that lower frequencies present greater wavelengths and therefore, ensure the reliable transmission of information contained in calls (Amorim et al., 2016). Additionally, turbidity was found to significantly influence Guiana dolphin whistle amplitude, where relative amplitude decayed with lower concentrations of dissolved oxygen and higher water turbidity (Deconto et al., 2021).

Finally, we found that pool dimensions did not appear to influence call parameters, where duration was only found to be significantly different between pools for younger male calls. Forrest (1993) states that long-range communication in shallow water is likely limited to <15m. The results of our study reinforce this idea, since the call parameters were more strongly degraded from 16-32m in shallow natural pools (maximum depth 1.58m). Although pool dimension did not affect call propagation over distance, the sediment of an environment plays an important role in sound propagation. Muddy sediment is highly reflective, and studies have shown, through comparisons between habitats, that the lowest levels of attenuation occur in habitats with muddy sediment (Forrest, 1993; Briggs & Erisman, 2021). Particularly, the sediment of the reintroduction enclosures is defined as sandy-litho-clastic and a potential retainer of organic material and terrigenous mud (Paiva, 2018). Thus, we can assume that Antillean manatee calls suffer little degradation caused by reflection from the substrate in their habitats and rather suffer propagation loss caused by turbidity (Deconto et al., 2021). This may further explain the lack of call degradation observed in this study, since the water was relatively clear during the experiments, and the sediments of the study natural pools were a mixture of sand and limestone/quartz and were, therefore, likely highly reflective. The reintroduction manatees in our study inhabit a mangrove environment on the Riacho Tabatinga, a tributary of the Tatuamunha estuary. Thus, this environment experiences high fluctuations in turbidity depending on tide level, i.e., high tide - low turbidity, low tide – high turbidity. It is, therefore, likely that manatee calls are adapted structurally to cope with their ever-changing environment, ensuring the greatest information transmission success under different environmental contexts (Chapter 4.1 of this thesis).

6.5 Conclusions

Here we found that Antillean manatee calls were relatively stable over distance and varying abiotic conditions, suggesting that manatees have adapted their vocal production and call structure to their habitats. Thus, from our data, we can infer that it is unlikely that the predicted increases in temperatures and pH caused by climate change will strongly affect the vocal communication of Antillean manatees, since this subspecies is ecologically highly adapted to abiotic fluctuations in their environment, including their vocal communication. However, the predicted increases in storm occurrence and sea level caused by climate change may impact Antillean manatee vocal communication more strongly in North-eastern Brazil. Therefore, we suggest that further studies should be carried out, investigating the impacts of turbidity on Antillean manatee call propagation, especially since estuary turbidity is directly affected by anthropogenic impacts, such as runoff, as well as natural events, such as storms and flooding. Also, calls recorded from free-living individuals should be used to further test the propagation properties of calls from Antillean manatees.

7 Final Considerations

7.1 Literature review

- We gathered 136 papers on the effects of climate change on marine mammal behavioural ecology and vocal communication.
- The greatest number of studies were published in 2018.
- The studies included a total of 41 marine mammal species.
- CiteScores were higher for papers investigating climate change and marine mammal behavioural ecology compared to papers investigating underwater sound propagation and marine mammal vocal communication.
- There is a distinct lack of information available on the effects of abiotic variables on marine mammal vocal communication.

7.2 Articles 1 and 2

- Antillean manatee groups produce variants of different call types which may be related to sex, age and individual.
- We describe a new call type: Pulse calls.
- Manatees produce calls that likely contain information about individual identity.
- Mother-calf pairs produce vocalisations that are structurally specific to the pair which may facilitate mother-calf recognition.

7.3 Articles 3 and 4

- Call rates differ between manatee groups during the day (5am-9pm) but not during the night (9pm-5am).
- Call rates also differ between groups during different tide regimes.
- Each group produced significantly more Squeak calls compared to other calls.
- Call rates differed when comparing captive and reintroduction animals.
- Tide only influenced the call rates of the older reintroduction males.
- Time of day influenced the call rates of the captive manatees and the younger reintroduction males.
- Neither time of day nor tide level affect the reintroduction pair, likely due to the high level of stress they were experiencing.
- Call structure differed for duration, frequency of maximum energy, maximum frequency and minimum frequency however, not enough to successfully differentiate the calls produced during different times of the day or tide levels.

- Manatee calls experience little degradation over distance and under different environmental conditions.
- Manatee calls are well-adapted to their environment and other factors such as water turbidity, may influence call propagation more strongly.

8 References

Affatati, A., Scaini, C. and Salon, S. 2022. Ocean sound propagation in a changing climate: Global sound speed changes and identification of acoustic hotspots. Earth's Future, 10, e2021EF002099.

Alencar, A.E.B. 2010. Caracterização de batimétrica, sedimentológica e geoquímica do estuário do rio Mamanguape – PB. Masters Dissertation. Universidade Federal de Pernambuco, Programa de Pós-Graduação em Geonciências.

Alves, M.D.O., Schwamborn, R., Borges, J.C.G., Marmontel, M., Costa, A.F., Schettini, C.A.F. & Araujo, M.E. 2013. Aerial survey of manatees, dolphins and sea turtles off northeastern Brazil: Correlations with coastal features and human activities. Biol Conserv. 161: 91-100.

Ames, A.E., Blackwell, S.B., Tevo, O.M. & Heide-Jørgensen, M.P. 2021. Evidence of stereotyped contact call used in narwhale (*Monodon monoceros*) mother-calf communication. PLoS ONE 16(8): e0254929.

Amorim, T.O.S., Andriolo, A., Reis, S.S., dos Santos, M.E. 2016. Vocalisations of Amazon river dolphins (*Inia geoffrensis*): characterisation, effect of physical environment and differences between populations. J Acoust Soc Am 139(3):1285-1293.

Anderson, M. J.; Gorley, R. N.; Clarke, K. 2008. R. PERMANOVA+ for PRIMER: guide to software and statistical methods. Plymouth: PRIMER-E.

Au, W.W. and Hastings, M.C. 2008. Principles of marine bioacoustics (Vol. 510). Springer.

Balensiefer, D.C., Attademo, F.L.N., Sousa, G.P., Freire, A.C.B., da Cunha, F.A.G.C., Alencar, A.E.B., Silva, F.J.L., Luna, F.O. 2017. Three decades of Antillean manatee (*Trichechus manatus manatus*) stranding along the Brazilian coast. Trop Cons Sci 10:1-9.

Barbieri, M.M. 2005. Physiological and behavioural thermoregulation in bottlenose dolphins (*Tursiops truncatus*) in Sarasota, Florida. M.Sc. Thesis, University of North Carolina, Wilmington, NC.

Barlow, D.R., Fournet, M. & Sharpe, F. 2019. Incorporating tides into the acoustic ecology of humpback whales. Mar Mamm Sci 35(1):11-18.

Baumgartner, M.F. & Fratantoni, D.M. 2008. Diel periodicity in both sei whale vocalisation rates and the vertical migration of their copepod prey observed from ocean gliders. Limnol Oceanogr, 53:2197-2209.

Berger-Tal, O., Wong, B.B.M., Candolin, U. & Barber, J. 2019. What evidence exists on the effects of anthropogenic noise on acoustic communication in animals? A systematic map protocol. Envrion Evidence. 8(1): 18.

Bindoff, N.L., Cheung, W.W.L., Kairo, J.G., Arístegui, J., Guinder, V.A., Hallberg, R., Hilmi, N., Jiao, N., Karim, M.S., Levin, L., O'Donoghue, S., Purca Cuicapusa, S.R., Rinkevich, B., Suga, T., Tagliabue, A., Williamson, P. 2019. Changing Ocean, Marine Ecosystems, and Dependent Communities. In: IPCC Special Report on the Ocean and Cryosphere in a Changing Climate [Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B, Weyer NM (eds.)].

Boness, D.J. & Bowen, W.D. 1996. The evolution of maternal care in pinnipeds: New findings raise questions about the evolution of maternal feeding strategies – BioScience. 46(9):645-654.

Bono, S., Kimura, S.S., Teoh, Z.Y., Ng, J.E., Ichikawa, K. & Ponnampalam, L.S. 2022. Whistle variation in Indo-Pacific humpback dolphins (*Sousa chinensis*) in relation to behavioural and environmnetal parameters northwestern peninsular Malaysia. Acoustics Australia. 50:315-329.

Bouchet, H., Blois-Heulin, C. & Lemasson, A. 2013. Social complexity parallels vocal complexity: a comparison of three non-human primates. Psychol. 4: 390.

Bowater, R.O., Norton, J., Johnson, S., Hill, B., O'Donaghue, P., Prior, H. 2003. Toxoplasmosis in Indo-Pacific humpbacked dolphins (*Sousa chinensis*), from Queensland. Aust Vet J 81:627-632.

Brady, B., Hedwig, D., Trygonis, V. & Gerstein, E. 2020. Classification of Florida manatee (*Trichechus manatus latirostris*) vocalizations. J. Acoust. Soc. Am.: 147(3): 1597-1606.

Brady, B., Moore, J. & Love, K. 2021. Behavior related vocalizations of the Florida manatee *(Trichechus manatus latirostris*). Mar Mamm Sci. 2021:1-5.

Briggs, C.R. & Erisman, B.E. 2021. Transmission loss of fish spawning vocalisations and the detection range of passive acoustic sampling in very shallow estuarine environments. Estuaries and Coasts. https://doi.org/10.1007/s12237-021-00914-5

Bucher, T.L., Ryan, M.J. & Bartholomew, G.A. 1982. Oxygen consumption during resting, calling and nest building in the frog *Physalaemus pustulosus*. Physiol. Zool. 55(1): 10-22.

Bradbury, J.W. and Vehrencamp, S.L. 1998. *Principles of animal communication*. Sunderland, MA: Sinauer.

Brodie J, Waterhouse J. 2012. A critical review of environmental management of the 'not so Great' Barrier Reef. Estuar Coast Shelf Science 104-105:1-22.

Buder, E. H., Chorna, L.B., Oller, D.K. & Robinson, R.B. 2008. Vibratory regime classification of infant phonation. J. Voice. 22(5): 553-564.

Cai, W., Borlace, S., Lengaigne, M, van Rensch, P., Collins, M., Vecchi, G…Jin, F-F. 2014. Increasing frequency of extreme El Niño events due to greenhouse warming. Nature Climate Change, 4(2): 111.

Caldwell, M.C. & Caldwell, D.K. 1965. Individualised whistle contours in bottlenosed dolphins *(Tursiops truncatus)* – Nature. 207:434-435.

Caldwell, M.C. & Caldwell, D.K. 1979. The whistle of the Atlantic bottlenosed dolphin (*Tursiops truncatus*)—ontogeny. In: Behaviour of marine animals (Winn, H.E. ed.). New York, Plenum Press; p. 369–401.

Caldwell, M.C., Caldwell, D.K., Tyack, P.L. 1990. Review of the signature whistle hypothesis for the Atlantic bottlenose dolphin. In Leatherwood S, Reeves R R (eds) The Bottlenose Dolphin. Academic, San Diego, pp. 199 –234.

Cann, K., Thomas, D.R., Salmon, R., Wyn-Jones, A. and Kay, D. 2013. Extreme water-related weather events and waterborne disease. Epidemiology & Infection, 141(4):671-686.

Caorsi, V.Z., Both, C., Cechin, S., Antunes, R. & Borges-Martins, M. 2017. Effects of traffic noise on the calling behaviour of two Neotropical hylid frogs. PLoS ONE 13(5): e0197632.

Charlton, B.D., Martin-Wintle, M.S., Owen, M.A., Zhang, H. & Swaisgood, R.R. 2018. Vocal behaviour predicts mating success in giant pandas. R. Soc. Open. Sci. 5: 181323.

Charrier, I, Mathevon, N, Jouventin, P. 2003. Individuality in the voice of fur seal females: an analysis study of the pup attraction call in *Arctocephalus tropicalis*. Mar Mamm Sci 19(1):161-172.

Charrier I, Harcout RG (2006) Individual vocal identity in mother and pup Australian sea lions (*Neophoca cinera*). J Mamm 87(5): 929-938.

Charrier, I., Aubin, T. & Mathevon, N. (2010). Mother-calf vocal communication in Atlantic walrus: a first field experimental study. Anim Cog 13: 471-482.

Charrier I, Marchesseau S, Dendrinos P, Tounta E, Karamanlidis AA (2017) Individual signatures in the vocal repertoire of the endangered Mediterranean monk seal: perspectives for population monitoring. Endang Sp Res, 32: 459-470.

Chavarría, M. R., Castro, J. & Camacho, A. 2015. The relationship between acoustic habitat, hearing and tonal vocalizations in the Antillean manatee (*Trichechus manatus manatus,* Linnaeus, 1758). Biol Open. 4:1237-1242.

Chaverri, G., Sandoval-Herrera, N. I., Iturralde-Pólit, P., Romero-Vásquez, A., Chaves-Ramírez & Sagot, H. (2021). The energetics of social signalling during roost location in Spix's disc-winged bats. J. Exp. Biol. 224(14): jeb238279.

Chen, N., Krom, M.D., Wu, Y., Yu, D. and Hong, H. 2018. Storm induced estuarine turbidity maxima and controls on nutrient fluxes across river-estuary-coast continuum. Science of the Total Environment, 628-629:1108-1120.

Cheney, D.L. & Seyfarth. R.M. 2018. Flexible usage and social function in primate vocalisations – PNAS. 115(9):1974-1979.

Cholewiak, D., Clark, C.W., Ponirakis, D., Frankel, A., Hatch, L.T., Risch, D., Stanistreet, J.E., Thompson, M., Vu, E. and van Parijs, S.M. 2018. Communicating amidst the noise: modelling the aggregate influence of ambient vessel noise on baleen whale communication space in a national marine sanctuary. Endang Sp Res, 36(5):59-75.

Civelek, C. 2013. The frequency of vocalisations exhibited by rescued Florida manatees (Trichecus manatus latirostris): the influence of time of day, sex and feeding. Technical Report, Miami Sequarium, Key Biscayne, FL.

Clark, C.W. 1983. Communication and behavior of the Southern Right Whale *(Eubalaena australis)*. In Payne, R (ed) Communication and Behavior of Whales AAAS Selected Symposium 76. Westview Press; Boulder, Colorado, pp 163-198.

Clausen, K.T., Wahlberg, M., Beedholm, H., Deruiter, S., Madsen, P.W. 2011. Click communication in harbour porpoises, *Phocoena phococena*. Bioacous 20: 1-28.

Collins, K.T., Terhune, J.M., Rogers, T.L., Wheatley, K.E., Harcourt, R.G. 2006. Vocal individuality of in-air weddell seal (*Leptonychotes weddellii*) pup "primary" calls. Mar Mamm Sci 22(4): 933-951.

Costa, A.F. 2006. Distribuição especial e *status* do peixe-boi marinho, *Trichechus manatus manatus,* (Sirenia: Trichechidae) no litoral leste do Estado do Ceará. Masters Dissertation, Universidade Federal do Ceará, Fortaleza.

Costa, B., Umeed, R., Attademo, F., Normande, I.C. & Bezerra, B. 2018. Comportamento vocal noturno e diurno do peixe-boi marinho (*Trichechus mantus manatus*) – IV Simpósio Pernambucano de Ecologia, Universidade Rural Federal de Pernambuco, Recife, Brazil.

Crawford, J.A., Quakenbush, L.T. and Citta, J.T. 2015. A comparison of ringed and bearded seal diet, condition and productivity between historical (1975-1984) and recent periods in the Alaskan Bering and Chukchi seas. Prog Oceanogr, 136:133-150.

Crockford, C. & Boesch, C. 2003. Context specific calls in wild chimpanzees, *Pan troglodytes verus*: Analysis of barks – Anim. Behav. 66:115–125.

Dall, S.R., Giraldeau, L.A., Olsson, O., McNamara, J.M. & Stephens, D.W. 2005. Information and its use by animals in evolutionary biology. Trends Ecol Evol. 20(4):187-193.

Davidson, A.D., Boyer, A.G., Kim, H., Pompa-Mansilla, S., Hamilton, M.J., Costa, D.P., Ceballos, G. and Brown, J.H. 2011. Drivers and hotspots of extinction risk in marine mammals. PNAS, 109(9):3395-3400.

Deconto, L.S., Freitas, T.C., Guaraldo, F.A.C., Leão, D., T., Silva, F. J. L. and Monteiro-Filho, E.L.A. 2021. Propagtion of Guiana dolphin sounds in their habitat: measured transmission loss and influence of environmental factors. Bioacoustics, DOI: 10.1080/09524622.2021.1929488.

Dechmann, D.K.N., Wikelski, M., van Noordwijk, H.J., Voigt, C.C. & Voigt-Heucke, S. 2013. Metabolic costs of bat echolocation in a non-foraging context support a role in communication. Front. Physiol. 4: 66.

De Figueiredo, L.D., Simão, S.M. 2009. Possible occurrence of signature whistles in a population of *Sotalia guianensis* (Cetacea, Delphinidae) living in Sepetiba Bay, Brazil. J Acoust Soc Am, 126: 1563.

De Vincenzi, G., Parisi, I., Torri, M., Papale, E., Mazzola, S., Nuth, C. and Buscsaino, G. 2019. Influence of environmental parameters on the use and spatiotemporal distribution of the vocalisations of bearded seals (*Erignathus barbatus*) in Kongsfjorden, Spitsbergen.

Dias, C. B. 2005. Dinâmica do sistema estuarino Timonha/Ubatuba (Ceará-Brasil): considerações ambientais. M.Sc. thesis. Federal University of Ceará, Brazil.

Dodson, S., Abrahms, B., Bograd, S.J., Fietcher, J., Hazen, E.L. 2020. Disentangling the biotic and abiotic drivers of emergent migratory behaviour using individual-based models. Ecological Modelling 432, 109225.

Dunlop, R.A., Cato, D.H., Noad, M.J. 2008. Non-song acoustic communication in migrating humpback whales (*Megaptera novaengliae*). Mar Mamm Sci 24(3): 613-629.

Dunn, C., Claridge, D., Durban, J., Shaffer, J., Moretti, D., Tyack, P.L. & Rendell, L.E. 2017. Insights into Blainville's beaked whale (*Mesoplodon densirostris*) echolocation ontogeny from recordings of mother-calf pairs – Mar Mam Sci. 33:1-18.

Edds, P.L., Tershy, B.R., Odell, D.K. 1993. Vocalizations of a captive juvenile and free-ranging adult-calf pairs of Bryde's whales, *Balaenoptera edeni*. Mar Mamm Sci 9:269- 284.

Evans, W. E., & Awbrey, F. T. 1988. Natural history aspects of marine mammal echolocation: Feeding strategies and habitat. In P. E. Nachtigall & P. W. B. Moore (Eds.), *Animal sonar: Processes and performance* (pp. 521- 534). New York: Plenum Press.

Favero, I.T., Favero, G.E., Choi-Lima, K.F., dos Santos, H.F., Souza-Alves, J.P., Silva, J.S. & Feitosa, J. 2020. Effects of freshwater limitation on distribution patterns and habitat use of the West Indian manatee, *Trichechus manatus*, in the northern Brazilian coast. Aquat Cons. 30(8): 1665-1673.

Fearey, J., Elwen, S.H., James, B.S. & Gridley, T. 2019. Identification of potential signature Trills from free-ranging common dolphins (*Delphinus delphis*) in South Africa - Anim Cog 22:777-789.

Ferguson, S., Stirling, I. and McLoughlin, P. 2005. Climate change and ringed seal (*Phoca hispida*) recruitment in western Hudson Bay. Mar Mam Sci, 21(1):121-135.

Figueiredo, L.D., Simão, S.M. 2014. Bryde's whale (*Balaenoptera edeni*) vocalisations from Southeast Brazil. Aquat Mamm 40(3): 225-231.

Fitch, W., Neubauer, J., & Herzel, H. 2002. Calls out of chaos: The adaptive significance of nonlinear phenomena in mammalian vocal production. Animal Behaviour 63: 407-418.

Forcada, J., Trathan, P.N., Boveng, P.L., Boyd, I.L., Burns, J.M., Costa, D.P., Fedak, M., Rogers, T.L., Southwell, C.J. 2012. Responses of Antarctic pack-ice seals to environmental change and increasing krill fishing. Biol Conserv 149:40-50.

Forrest, T.G., Miller, G.L., Zagar, J.R. 1993. Sound propagation in shallow water: Implications for acoustic communication by aquatic animals. Int J Anim Sound Rec 4: 259-270.

Freeberg, T.M., Dunbar, R.I.M. & Ord, T.J. 2012. Social complexity as a proximate and ultimate factor in communicative complexity. Philos Trans R Soc Lond B Biol Sci. 367(1597):1785-1801.

Fripp, D., Owen, C., Quintana-Rizzo, E., Shapiro, A., Buckstaff, K., Jankowski, K., Wells, R., Tyack, P. 2005. Bottlenose dolphin (*Tursiops truncatus*) calves appear to model their signature whistles on the signature whistles of community members. Anim Cogn 8: 17–26.

Fripp, D. & Tyack, P.L. 2008. Postpartum whistle production in bottlenose dolphins - Mar Mam Sci. 24(3):249-505.

Fuentes, M.M.P.B., Delean, S., Grayson, J., Lavender, S., Logan, M., & Marsh, H. 2016. Spatial and temporal variation in the effects of climactic variables on dugong calf production. -PloS ONE. 11(6):e0155675.

Fury, C.A., Harrison, P.L. 2011. Impact of flood events on dolphin occupancy patterns. Mar Mamm Sci 27:185-205.

Garcia-Rodriguez, A.I., Bowen, B.W. Domning, D., Mignucci-Giannoni, A.A., Marmontel, M., Montoya-Ospina, R.A., Morales-Vela, B., Rudin, M., Bonde, R.K. & McGuiress, P.M. 1998. Phylogeography of the West Indian manatee (*Trichechus manatus*): how many populations and how many taxa? Mol Ecol 7:1137-1149.

Gazioglu, C., Müftüoglu, A.E., Demir, V., Aksu, A., Okutan, V. 2015 Connection between ocean acidification and sound propagation. Int J Environ Geoinformatics 2(2):16-26.

George, J.C., Druckenmiller, M.L., Laidre, K.L., Suydam, R and Person, B. 2015. Bowhead body condition and links to summer sea ice and upwelling in the Beaufort Sea. Prog Oceanogr, 136:250-262.

Geraci, J.R., Andersen, D.M., Timperi, R.J., St. Aubin, D.J., Early, G.A., Prescott, J.H., Mayo, C.A. 1989. Humpback whales fatally poisoned by dinoflagellate toxin. Can J Fish Aquat Sci 46:1895-1898.

Gerstein, E., Gerstein, L., Blue, J. 2008. Ultrasonic hearing and vocalisations are used in communication by West Indian manatee mothers and calves. J Acoust Soc Am 124: 2549.

Gill, L.F., Goymann, W., Maat, A.T. & Gahr, M. 2015. Patterns of call communication between group-housed zebra finches change during the breeding cycle. eLife. 4:e07770.

Gómez-Carrasco, G., Lesher-Gordillo, J.M., Olivera-Gómez, L.D., Bonde, R.K., Arriaga-Weiss, S., Hernández-Martínez, R., Castañón-Nájera, G., Jiminéz-Domínguez, D., Romo-López, A. & Delgado-Estrella, A. 2020. Genetic diversity and structure from Antillean manatee (*Trichechus manatus manatus*) in the Southern Gulf of México: Comparison between connected and isolated populations. *Trop Conserv Sci.* 11(1):2018.

Goold, J.C. 2006. A diel pattern in vocal activity of short-beaked common dolphins, *Delphinus delphis*. Mar Mamm Sci 16(1):240-244.

Green, S.M. 1981. Sex differences and age gradations in vocalisations of Japanese and lion-tailed monkeys (*Macaca fuscata* and *Macaca 160runcat*). – Anim Zool. 21:165-183.

Gridley, T., Cockcroft, V.G., Hawkins, E.R., Blewitt, M.L., Morisaka, T., & Janik, V.M. 2014. Signature Trills in free-ranging populations of Indo-Pacific bottlenose dolphins, *Tursiops aduncus*. – Mar Mam Sci. 30:1-16.

Gulland, F., and Hall, A. 2007. Is marine mammal health deteriorating? Trends in the global reporting of marine mammal disease. Ecohealth, 4(2): 135-150.

Gustison, M.L., le Roux, A. & Bergman, T.J. 2012. Derived vocalisations of geladas *(Theropithecus gelada)* and the evolution of vocal complexity in primates. Phil. Trans. R. Soc. B. 367(1597): 1847-1859.

Gustison, M.L. & Bergman, T.J. 2016. Vocal complexity influences female responses to gelada male calls. Sci. Reports. 6: 19680.

Halliday, T.R. and Slater, P.J.B. 1983. Introduction. In T. R. Halliday & P. J. B. Slater (Eds.), *Animal behav- iour: Communication* (Vol. 2, pp. 1-8). Oxford, UK: Blackwell Scientific Publications.

Hamilton, C.D., Vacquié-Garcia, J., Kovacs, K.M., Ims, R.A., Kohler, J., Lydersen, C. 2019. Contrasting changes in space use induced by climate change in two Arctic marine mammal species. Biol Lett 15:1-4.

Hanna, D.E.L., Wilson, D.R., Blouin-Demers, G. & Mennill, D.J. 2014. Spring peepers *Pseudacris crucifer* modify their call structure in response to noise. Current Zool. 60(4): 438-448.

Hartman, D.S. 1979. Ecology and behaviour of the manatee, *Trichechus manatus*, in Florida (Special Publication 5). New York: American Society of Mammologists.

Harvell, C., Kim, K., Burkholder, J., Colwell, R., Epstein, P.R., Grimes, D, Overstreet, R.M. et al.1999. Emerging marine disease-climate links and anthropogenic factors. Sci, 285:1505-1510.

Harvell, C., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., and Samuel, M.D. 2002. Climate warming and disease risks for terrestrial and marine biota. Science, 296:2158-2162.

Hastings, R.A., Rutterford, L.A., Freer, J.J., Collins, R.A., Simpson, S.D. and Genner, M.J. 2020. Climate change drives poleward increases and equatorward decreases in marine species. Current Biology, 30, 1572-1577.

Hénaut, Y., Lopez, S.P.B., Machkour-M'Rabet, S., Morales-Vela. B., Winterton, P., & Delfour, F. (2010). Activities and social interactions in captive Antillean manatees in Mexico. Mammalia. 74:141-146.

Hernández, M., Robinson, I., Aguilar, A., González, L.M., López-Jurado, L.F., Reyero, M.I., Cacho. E., Franco, J., López-Rodas, V., Costas, E. 1998. Did algal toxins cause monk seal mortality? Nature 393:28. ·

Hettiarachchi, S., Wasko, C. and Sharma, A. 2018. Increase in flood risk resulting from climate change in developed urban watershed – the role of storm temporal patterns. Hydrol Earth Syst Sci, 22: 2041-2056.

Hildebrand, J.A. 2009. Anthropogenic and natural sources of noise in the ocean. Mar Ecol Prog Ser 395:5-20.

Holt, M.M., Dunkin, R.C., Noren. D., & Williams, T.M. 2013. Are there metabolic costs of vocal responses to noise in marine mammals? J. Acoust. Soc. Am. 133(5): 3536.

Holt, M.M., Noren, D., Dunkin, R.C. & Williams, T. M. 2015. Vocal performance affects metabolic rate in dolphins: implications for animals communicating in noisy environments. J Exp. Biol. 00:1-8.

Holt, M. M., Noren, D. P., Dunkin, R. C., and Williams, T. M. 2016. Comparing the metabolic costs of different sound types in bottlenose dolphins. Proc. Meet. Acoust. 27:010019.

Huang, W., Wang, D. & Ratilal, P. 2016. Diel and spatial dependence of humpback song and non-song vocalistions in fish spawning grounds. Rem Sens 8(9):712.

Huntington, H.P., Quakenbush, L.T. and Nelson, M. 2017. Evaluating the effects of climate change on indigenous marine mammal hunting in northern and western Alaska using traditional knowledge. Front Mar Sci 4:319.

Ilyina, T., Zeebe, R.E., Brewer, P.G. 2009. Future ocean increasingly transparent to low-frequency sound owing to carbon dioxide emissions. Nature Geoscience 3:18-22.

Indeck, K.L. 2020. Acoustic communication of female-calf humpback whales during migration. Undergraduate thesis, Unviersity of Queensland, Australia.

IPCC. 2001. Climate Change 2001: The scientific basis. Contribution of Working Group I to the Third assessment report of the Intergovernmental Panel on Climate Change (IPCC) [Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA (eds)]. Cambridge University Press: Cambridge, UK, and New York, USA

IPCC. 2013. Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

IPCC. 2014. Climate change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field CB, Barros VR, Dokken DJ, Mach KJ, Mastrandrea MD, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, White LL (eds)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

IPCC. 2022. Climate change 2022: Impacts adaptation and vulnerability. Contribution of working group II to the sixth assessment report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.

Johnson, G.C. and Lyman, J.M. 2020. Warming trends increasingly dominate global ocean. Nature Climate Change, 10(8):757-761.

Johnson, D., Adelaide Ferreira, M., Kenchington, E. 2018. Climate change is likely to severely limit the effectiveness of deep sea ABMTs in the North Atlantic. Mar. Policy 87:111–122 doi:10.1016/j.marpol.2017.09.034.

Kastak, D., Southall, B.L., Schusterman, R.J. and Kastak, C.R. 2005. Underwater temporary threshold shift in pinnipeds: Effects of noise level and duration. J Acoust Soc Am. 11(5):3154-3163.

Kazial, K.A., Burnett, S.C., Masters, W.M. 2001. Individual and group variation in echolocation calls of big brown bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae). J Mamm 82(2): 339-351.

Kennedy, J.J., Rayner, N.A., Atkinson, C.P. and Killick, R.E. 2019. An ensemble data set of sea surface temperature change from 1850: The Met Office Hadley Centre HadSST.4.0.0.0 Data Set. J. Geophys. Res. D. Atmospheres *124*, 7719-7763.

Kelly, B.P. 2001. Climate change and ice breeding pinnipeds. In: Walther GR, Burga CA, Edwards P (eds) "Fingerprints" of climate change. Kluwer Academic/Plenum Publishers. New York 2001, pp. 43-55.

Kennedy, J.J., Rayner, N.A., Atkinson, C.P. & Killick, R.E. 2019. An ensemble data set of sea surface temperature change from 1850: The Met Office Hadley Centre HadSST.4.0.0.0 Data Set. J. Geophys. Res. D. Atmospheres *124*, 7719-7763.

Kerosky, S.M., Sirović, A., Roche, L.K., Baumann-Pickering, S., Wiggins, S.M., Hildebrand, J.A. 2012. Bryde’s whale seasonal range expansion and increasing presence in the Southern California Bight from 2000-2010. Deep Sea Research Part I: Oceanographic research papers. 52:125-132.

Kershaw, J.L., Ramp, C.A., Sears, R., Plourde, S., Brosset, P., Miller, P.O., Hall, A.J. 2021. Declining reproductive success in the Gulf of St. Lawrence’s humpback whales (*Megaptera novaeangliae*) reflects ecosystem shifts on their feeding grounds. Global change biology, 27(5), 1037-1041.

King, S.L., Guarino, E., Keaton, L., Erb, L., Jaakkola, K. 2016. Maternal signature whistle use aids mother-calf reunions in a bottlenose dolphin, *Tursiops truncatus*. Behav Proc 126:64-70

Kloepper, L.N., Simmons, A.M. 2014. Bioacoustic monitoring contributed to an understanding of climate change. Acoust Today 2014:8-15.

Krams, I., Krama, T., Freeberg, T.M., Kullberg, C. & Lucas, J.R. 2012. Linking social complexity and vocal complexity: a parid perspective. Phil. Trans. R. Soc. B. 367: 1879-1891.

Kremers, D., Jaramillo, M.B., Böye, M., Lemasson, A. & Hausberger, M. 2014. Nocturnal vocal activity in captive bottlenose dolphins (*Tursiops truncatus*): could dolphin have presleep choruses? Anim Behav Cog 1(4):464-469.

Kuiken, T., Kennedy, S., Barrett, T., Van de Bildt, M., Borgsteede, F., Brew, S…Eybatov, T. 2006. The 2000 canine distemper epidemic in Caspian seals (*Phoca capsica*): Pathology and analysis of contributory factors. Vet Path, 43(3): 321-338.

Lachenbruch, P.A., Goldstein, M. 1979. Perspectives in Biometery. Biometrics 35(1): 69-85.

La Manna, G., Rako-Gòspic, N., Manghi, M., Ceccherelli, G. 2019. Influence of environmental, social and behavioural variables on the whistling of the common bottlenose dolphin (*Tursiops truncatus*). Behav Ecol Sociobiol. 73:121.

Landrau-Giovanetti, N., Mignucci-Giannoni, A.A. & Reidenberg, J.S. 2014. Acoustical and Anatomical determination of sound production and transmission in West Indian (*Trichechus manatus*) and Amazonian manatees. Anatom Rec. 297:1896-1907.

Leaper, R., Cooke, J., Trathan, P., Reid, K., Rowntree, V., Payne, R. 2006. Global climate drives southern right whale (*Eubalaena australis*) population dynamics. Biol Lett 2:289-292

Leão, D.T., Monteiro-Filho, E.L.A. & Silva, F.J.L. 2016. Acoustic parameters of sounds emitted by *Sotalia guianensis*: dialects or acoustic plasticity. J Mammol, 97(2): 611:618

Learmonth, J.A., MacLeod, C.D., Santos, M.B., Pierce, G.J., Crick. H.Q.P., Robinson, R.A. 2006. Potential effects of climate change on marine mammals. Oceanogr Mar Biol: An Annual Review 44:43-464.

Lee, J.R., Raymond, B., Bracegirdle, T.J., Chadès, I., Fuller, R.A., Shaw, J.D., Terauds, A. 2017. Climate change drives expansion of Antarctic ice-free habitat. Nature 547: 49-67.

Lesage, V., Barrette, C., Kingsley, M.C.S. & Sjare, B. 1999. The effect of vessel noise on the vocal behaviour of belugas in the St. Lawrence river estuary, Canada. Mar Mamm Sci. 15(1): 65-84.

Lilly, J.C. 1963. Distress call of the bottlenose dolphin: Stimuli and evoked behavioural responses. Science, 139(3550): 116-118.

Lin, T-H., Akamatsu, T.& Chou, L-S. 2013. Tidal influences on the habitat use of Indo-Pacific humpback dolphins in an estuary. Mar Biol, 160:1353-1363.

Lingle, S., Wyman, M.T., Kotrba, R., Teichroeb, L.J., & Romanow, C.A. 2012. What makes a cry a cry? A review of infant distress calls. – Current Zool. 58(5):698-726.

Ljungblad, D.K., Leatherwood, S., Dahlheim, M.E. 1980. Sounds recorded in the presence of an adult and calf bowhead whale. Mar Fish Rev 42: 86-87.

Lucchini, K., Umeed, R., Guimarães, L., Santos, P., Sommer, I. & Bezerra, B. 2021. The role of touch in captive and semi-captive Antillean manatees (*Trichechus manatus manatus*). Behav. 158(3-4):291-313.

Luís, A.R., Couchinho, M.N. & dos Santos, M.E. 2014. Changes in the acoustic behaviour of resident bottlenose dolphins near operating vessels. Mar Mamm Sci. 30(4): 1417-1426.

Lynch, J.F., Gawarkiewicz, G.G., Lin, Y.T., Duda, T.F., Newhall, A.E. 2018. Impacts of ocean warming on acoustic propagation over continental shelf and slope regions. Oceanography 31(2):17-181. doi:10.5670/oceanog.2018.219

Mann, D.A., O'Shea, T.J., Nowacek, D.P. 2006. Nonlinear dynamics in manatee vocalisations. Mar Mamm Sci 22(3):548-555.

Marcoux, M., Whitehead, H., & Rendell, L. 2006. Coda vocalisations recorded in breeding areas almost entirely produced by mature female sperm whales (*Physeter macrocephalus*). – Canad J Zool. 84:609-614.

Marcoux, M., Ferguson, S.H., Roy, N., Bedard, J.M., Simard, Y. 2017. Seasonal marine mammal occurrence detected from passive acoustic monitoring in Scott Inlet, Nunavut, Canada. Polar Biology, 40:1127-1138.

McCordic, J.A., Root-Gutteridge, H., Cusano, D.A., Denes, S.L., Parks, S. 2016. Calls of North Atlantic right whales *Eubalaena glacialis*. Endang Spec Res 30: 157-169.

MacLeod, C.D. 2009. Global climate change, range changes and potential implications for the conservation of marine cetaceans: a review and synthesis. Endang Species Res 7:125-136.

Marley, S.A., Salgado Kent, C.P., Erbe, C., Parnum, I.M. 2017. Effects of vessel traffic and underwater noise on the movement, behaviour and vocalisations of bottlenose dolphins in an urbansied estuary. Science reports, 7:13437.

Matthews, J.N., Rendell, L.E, Gordon, J., MacDonald, D.W. 1999. A review of frequency and time parameters of cetacean tonal calls. Bioacoustics 10:47-71.

May-Collado, L., Agnarsson, I., Wartzok, D. 2007. Re-examining the relationship between body size and tonal signals frequency in whales: A comparative approach using a novel phylogeny. Mar Mamm Sci 23: 524-552.

Maxwell, S.M., Hazen, E.L., Bograd, S.J., Halpern, B.S., Breed, G.A., Nicketl, B., Teutschel, N.M., Crowder, L.B., Benson, S., Dutton, P.H., Bailey, H., Kappes, M.A., Kuhn, C.E., Weise, M.J., Mate, B., Shaffer, S.A., Hassrick, J.L., Henry, R.W., Irvine, L., McDonald, B.I., Robinson, P.W., Block, B.A., Costa, D.P. 2013. Cumulative human impacts on marine predators. Nat Commun 4:2688.

May-Collado, L., Agnarsson, I., & Wartzok, D. 2007. Re-examining the relationship between body size and tonal signals frequency in whales: A comparative approach using a novel phylogeny. – Mar Mam Sci 23:524-552.

Medeiros, I.S., Rebelo, V.A, Santos, S.S., Menezes, R., Almeida, N.V., Messias, L.T., Nascimento, J.L.X., Luna, F.O., Marmontel, M. & Borge, J.C.G. 2021. Spatiotemporal dynamics of mangrove forest and association with strandings of Antillean manatee (*Trichechus manatus*) calves in Paraíba, Brazil. J Mar Biol Assoc UK. 101(3):503-510.

Mercado III, E. and Frazer, L.N. 1999. Environmental constraints on sound transmission by humpback whales. J Acoust Soc Am. 106(5):3004.

Meirelles, A.C.O. 2008. Mortality of the Antillean manatee, *Trichechus manatus manatus*, in Ceará State, North-eastern Brazil. Mar Biol Assoc UK 88(6):1133-1137.

Merchan, F., Echevers, G., Poveda, H., Sanchez-Galan, J.E., & Guzman, H.M. 2019. Detection and identification of manatee individual vocalisations in Panamanian wetlands using spectrogram clustering. – J Acoust Soc Am. 146:1745-1757.

Menze, S., Zitterbart, D.P., van Opzeeland, I., Boebel, O. 2017. The influence of sea ice, wind speed and marine mammals on Southern Ocean ambient sound. R Soc Open Sci, 4:160370.

Miksis-Olds, J.L., Tyack, P.L. 2009. Manatee (*Trichechus manatus*) vocalization usage in relation to environmental noise levels. J Acoust Soc Am 125(3):1806-1815.

Miller, J.H., Kloepper, L.N., Potty, G.R., Spivack, A.J., D'Hondt, S.L., Turner, C. & Simmons, A.M. 2014. The effects of pH on acoustic transmission loss in an estuary. Proc Mtgs Acoust. 22: 00501.

Mishima, Y., Morisaka, T., Itoh, M., Matsuo, I., Sakaguchi, A., Miyamoto, Y. 2015. Individuality embedded in the isolation calls of captive beluga whales (*Delphinapterus leucas*). Zool Lett 1: 27.

Mitani, J.C., & Brandt, K.L. 1994. Social factors influence the acoustic variability in the long-distance calls of male chimpanzees. – Ethology. 96:233–252.

MMA. 2014. Portaria do Ministério do Meio Ambiente N° - 444 de 17 de dezembro de 2014 [Ordinance No. 444 of the Brazilian Ministry of the Environment, 17 December 2014].

Monsczak, A., Mueller, C., Miller, M.E., Ji, Y., Borgianni, S.A. & Montie, E.W. 2019. Sound patterns of snapping shrimp, fish and dolphins in an estuarine soundscape in the southeastern USA. MEPS, 609: 49-68.

Moore, S.E. & Ridgeway, S.H. 1996. Patterns of dolphin sound production and ovulation. Aquat Mamm. 22:175-184.

Moore, S.E., Huntington, H.P. 2008. Arctic marine mammals and climate change: impacts and resilience. Ecol. Appl 18, S157-S165.

Moore, S.E., Reeves, R.R. 2018. Tracking arctic marine mammal resilience an era of rapid ecosystem alteration. PLoS ONE 16(10):1-7.

Morisaka, T., Yoshida, Y., Akune, Y., Mishima, H., & Nishimoto, S. 2013. Exchange of "signature" calls in captive belugas (*Delphinapterus leucas*). – J Ethol. 31:141 149.

Moritz, R.E., Bitz, C.M., Steig, E.J. 2002. Dynamics of Recent Climate Change in the Arctic. Science 297:1497.

Murie, J. 1872. On the form and structure of the manatee (*Manatus americanus*). Trans Zool Soc Lond 8:127-202.

Naruba, A.D., Monczak, A., Balmer, B.C., Hart, L.B., Soueidan, J., Montie, E.W. 2021. Long-term passive acoustics to assess spatial and temporal vocalisations patterns of Atlantic common bottlenose dolphins (*Tursiops truncatus*) in the May River estuary, South Carolina. Mar Mamm Sci, 1-25.

Newman, J. 2004. The primate isolation call: A comparison with precocial birds and non-primate mammals. In: Rogers LJ, Kaplan G, editors. Comparative Vertebrate Cognition: Are Primates Superior to Non-primates? (Rogers, L.J, & Kaplan, G. eds.). New York: Kluwer Academic/ Plenum Publishers, p.171−187.

Newman, J.D. 2007. Neural circuits underlying crying and cry responding in mammals. - Behav Brain Res. 182:155− 165.

Nicol, S., Worby, A., Leaper, R. 2008. Changes in the Antarctic sea ice ecosystem: potential effects on krill and baleen whales. Mar Freshwater Res 35:371-374.

Noren, D.P., Holt, M.M., Dunkin, R.C., Williams, T.M. 2013. The metabolic cost of communicative sound production in bottlenose dolphins (*Tursiops truncatus*). J. Exp. Biol. 261(9): 1624-1629.

Nousek, A.E., Slater, P.J.B., Wang, C., & Miller, P.J.O. 2006. The influence of social affiliation on individual vocal signatures of northern resident killer whales. – Biol Lett. 2:481-484.

Nuñez, C.M.V. & Rubenstein, D.I. 2020. Communication is key: Mother-offspring signalling can affect behavioural responses and offspring survival in feral horses (*Equus caballus*). PLoS ONE. 15(4): e0231343.

Okabe, S., Nagasawa, M., Mogi, K. & Kikusui. T. 2012. The importance of mother–infant communication for social bond formation in mammals. Anim Sci J 83(6): 446–52.

Oliver, E.C.J., Donat, M.G., Burows, M.T., Moore, P.J., Smale, D.A., Alexander, L.V…Wernberg, T. 2018. Longer and more frequent marine heatwaves over the past century. Nature Communications, 9(1): 1324.

Oliveira, C., Wahlberg, M., Silva, M.A., Johnson, M., Antune, R., Wisniewska, D.M., Fais, A., Gonçalves, J., Madsen, P.T. 2015. Sperm whale codas may encode individuality as well as clan identity. J Acoust Soc Am 139(5): 2860-2869

Ophir, A.G., Schrader, S.B. & Gillooly. J.F. 2010. Energetic cost of calling: general constraints and species-specific differences. J Evol Biol 23(7): 1564-1569.

Opzeeland, I.V. 2010. Acoustic ecology of marine mammals in polar oceans. Reports on Polar and Marine Research, D-27570 BREMERHAVEN, Helmholtz Gemeinschaft.

Orgeret, F., Thiebault, A., Kovacs, K.M., Lydersen, C., Hindell, M.A., Thompson, S.A., Sydeman, W. and Pistorius, P.E. 2021. Climate change impacts on seabirds and marine mammals: the importance of study duration, thermal tolerance and generation time. Ecology Letters. 25(1):218-239.

O'Shea, T.J., & Poché, L.B. 2006. Aspects of underwater sound communication in Florida manatees (*Trichechus manatus latirostris*). – J Mammol. 87(6):1061–1071.

Oswald, J. N., Rankin, S., & Barlow, J. 2008. To whistle or not to whistle? Geographic variation in the whistling behavior of small odontocetes. Aquat Mamm. 34:288–302.

Otsuki, M., Akamatsu, T., Nobetsu, T. & Mitani, Y. 2021. Seasonal and diel changes in cetacean vocalisations monitored by passive acoustic methods in Nemuro Strait adjacent to the Shiretoko World Natural Heritage Site. Mar Mamm Sci 37(4):1330-1340.

Ouattara, K., Lemasson, A., & Zuberbühler, K. 2009. Campbell's monkeys concatenate vocalisations into context-specific call sequences. – Proc Nat Acad Sci USA. 106:22026–22031.

Paiva, L.M. 2018. Variabilidade estrutural da floresta de mangue do estuário do rio Tatuamunha, Porto de Pedras, Alagoas, Brasil. Dissertation, Universidade Federal de Pernambuco

Parks, S.E. & Clark, C.W. 2007. Short- and long-term changes in right whale calling behaviour: The potential effects of noise on acoustic communication. J Acoust Soc Am. 122(6): 3725

Parks, S.E., Cusano, D.A., Van Parijs, S.M., Nowacek, D.P. 2019. Acoustic crypsis in communication by North Atlantic right whale mother–calf pairs on the calving grounds. Biol Lett 15: 20190485.

Pine, M.K., Wilson, L., Jeffs, A.G., McWhinnie, L., Juanes, F., Scuderi, A. and Radford, C. 2021. A gulf in lockdown: how an enforced ban on recreational vessels increased dolphin and fish communication ranges. Glob Change Biol, 27:4839-4848.

Pitcher, B.J., Harcourt, R.G. & Charrier, I. 2012. Individual identity encoding and environmental constraints in vocal recognition of pups by Australian sea lion mothers. Animal Behav. 8: 681-690.

Podos, J., Southall, J.A. & Rossi-Santos, M.R. 2004. Vocal mechanics in Darwin's finches: correlation of beak gape and song frequency. J Exp Biol, 207:607-619.

Preen, A., Marsh, H. 1995. Response of dugongs to large-scale loss of seagrass from Hervey Bay, Queensland, Australia. Wildl Res 22:507-519.

Probert, R., Bastian, A., Elwen, S.H., James, B.S., & Gridley, T. 2021. Vocal correlates of arousal in bottlenose dolphins (*Tursiops* spp.) in human care. PLoS One 16(9): e0250913

Putland, R.L., Merchant, N.D., Farcas, A. and Radford, C.A. 2018. Vessel noise cuts down communication space for vocalising fish and marine mammals. Glob Change Biol. 24: 1708-1721.

Ramírez-Jiménez, H.H., Olivera-Gómez, L.D. & Cueva, H. 2017. Habitat use by the Antillean manatee (*Trichechus manatus*) during an extreme dry season in an urban lake in Tabasco, Mexico. Therya. 8(1): 3-18.

Ramos, Eric A., Maust-Mohl, M., Collom, K.A., Brady, B., Gerstein, E.R., Magnasco, M.O., Reis, D. 2020. The Antillean manatee produces broadband vocalizations with ultrasonic frequencies. J Acoust Soc Am: 147(2): EL80-EL86.

Rekdahl, M.L., Dunlop, R.A. & Noad, M.J. 2013. Temporal stability and change in the social call repertoire of migrating humpback whales. J Acoust Soc Am. 133(3): 1785-1795.

Reynolds III, JE (1981) Behaviour patterns in the West Indian manatee, with emphasis on feeding and diving. Florida Sci 44(4): 233-242.

Rice, A.N., Tielens, J.T., Estabrook, B.J., Muihead, C.A., Rahaman, A., Guerra, M. and Clark, C. 2014. Variation of ocean acoustic environments along the western North Atlantic coast: A case study in context of the right whale migration route. Ecol Inform, 21:89-99.

Risch, D., Wilson, S.C., Hoogerwerf, M., van Geel, N.C.F., Edwards, E.W.J., & Brookes, K.L. 2019. Seasonal and diel acoustic presence of North Atlantic minke whales in the North Sea. Sci Rep 9:3571.

Rendell, L., Cantor, M., Gero, S., Whitehead, H., & Mann, J. 2019. Causes and consequences of female centrality in cetacean societies. – Phi Trans R Soc B. 374:20180066.

Royal Society. 2005. Oceanic acidification due to increasing atmospheric carbon dioxide. The Royal Society, The Clyvedon Press Lts, Cardiff, UK.

Rycyk, A.M., Factheu, C., Brady, B.A., Kikuchi, M., Nations, H.F., Kapfer, K., Hampton, C.M., Garcia, E.R. & Kamla, A.T. 2021. First characterisation of vocalisations and passive acoustic monitoring of the vulnerable African manatee (*Trichechus senegalensis*). J Acoust Am Soc, 150:3028.

Saloma, A. 2018. Humpback whales (Megaptera novaeangliae) mother-calf interactions. Thesis, Université Paris Saclay (COmUE); Université d'Antananarivo.

Sanderson, C.E. and Alexander, K.A. 2020. Unchartered waters: Climate change likely to intensify infectious disease outbreaks causing mass mortality events in marine mammals. Glob Change Biol, 26:4284-4301.

Santos, S.S., Medeiros, S.I., Rebelo, V.A., Carvalho, A.O.B., Debut, J.P., Mantovani, J.E., Círiaco, R.D., Santos, R.E.G, Marmontel, M., Noramande, I.C., Velôsos, T.M.G. & Borges, J.C.G. 2021. Home ranges of released West Indian manatees *Trichechus manatus*. Oryx. 1-8.

Sadykova, D., Scott, B.E., de Dominicis, M., Wakelin, S.L., Wold, J. and Sadykov, A. 2019. Ecological costs of climate change on marine predator-prey population distributions by 2050. Ecol Evol. 10:1069-1086.

Sauvé, C.C., Beauplet, G., Hammil, M.O. & Charrier, I. 2015. Acoustic analysis of airborne, underwater and amphibious mother attraction calls by wild harbour seal pups (*Phoca vitulina*).

Sayigh S, Tyack PL, Wells, RS, Scott MD. 1990. Signature whistles of free-ranging bottlenose dolphins, *Tursiops truncatus*: stability and mother-offspring comparisons. Behav Ecol Sociobiol 26: 247-260.

Sayigh, L.S., Tyack, P.L., Wells, R.S., Scott, M.D, Irvine, A.B. 1995. Sex difference in signature whistle production of free-ranging bottlenose dolphins *Tursiops truncatus*. Behav Ecol Sociobiol 36: 171–177.

Schofield, O., Ducklow, H.W., Martinson, D.G., Meredith, M.P., Moline, M.A., Fraser, W.R. 2010. How Do Polar Marine Ecosystems Respond to Rapid Climate Change? Science 328:1520-1523.

Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., Cordaor, J., DeLong, R., De Vogelaere, A., Harvey, J. et al. 2000. Mortality of sea lions long the central Californian coast linked to a toxic diatom bloom. Nature, 403:80-84.

Schumann, N., Gales, N.J., Harcourt, R.G., Arnould, J.P.Y. 2013. Impacts of climate change on Australian marine mammals. Australian Journal of Zoology 61(2):146-160. doi:10.1071/ZO12131

Seghal, A., Tumar, I. & Schönwälder, J. 2010. Effects of climate change and anthropogenic ocean acidification on underwater acoustic communications. OCEANS'10 IEEE SYDNEY, 24-27 May 2010, Sydney, NSW, Australia. doi:10.1109/OCEANSSYD.2010.5603511

Shabangu, F.W., Yemane, D., Stafford, K.M., Ensor, P., Findlat, K.P. 2017. Modelling the effects of environmental conditions on the acoustic occurrence and behaviour of Antarctic blue whales. PLoS ONE, 12(2):e0172705.

Shapiro, A.D. 2006. Preliminary evidence for signature vocalisations among free-ranging narwhals (*Monodon monoceros*). – J Acoust Soc Am. 120(3):1695-1705.

Simeone, C.A., Gulland, F.M., Norris, T. and Rowles, T.K. 2015. A systematic review of changes in marine mammal health in North America, 1972-2012: The need for a novel integrated approach. PLoS One, 10(11), e0142105.

Simon, M., Stafford, K.L., Beedholm, K., Lee, C.M., Madsen, P.T. 2010. Singing behaviour of fin whales in the Davis Strait with implications for mating, migration and foraging. J Acoust Soc Am. 128(5): 3200-3210.

Simmonds, M.P., Issac, S.J. 2007. The impacts of climate change on marine mammals: early signs of significant problems. Oryx 41(1):19-26.

Simmonds, M.P., Eliott, W.J. 2009. Climate change and cetaceans: concerns and recent developments. J Biol Ass UK 89(1):203-210.

Slocombe, K.E., Townsend, S.W. & Zuberbühler, K. 2009. Wild chimpanzees (*Pan troglodytes schweinfurthii*) distinguish between different scream types: Evidence from a playback study. Anim. Cog. 12: 441–449.

Sousa-Lima, R.S., Paglia, A.P., Da Fonseca, G.A.B. 2002. Signature information and individual recognition in the isolation calls of Amazonian manatees, *Trichechus inunguis* (Mammalia: Sirenia). – Anim Behav. 63(2):301–310.

Sousa-Lima, R.S., Paglia, A.P., & da Fonseca, G.A.B. 2008. Gender, age, and identity in the isolation calls of Antillean manatees (*Trichechus manatus manatus*). – Aquat Mam. 34(1):109–122.

Stafford, K.M., Castellote, M. and Guerra, Berchock. 2018. Seasonal acoustic environments of beluga and bowhead whale core-use regions in the Pacific Arctic. Deep Sea Research Part II: Tropical Studies in Oceanography, 152: 108-120.

Stienessen, S. C. 1998. Diel, seasonal, and species- specific trends in vocalizations of dolphins in the Gulf of Mexico. Master of Science thesis, Texas A&M University, Galveston. 73 pp.

Sunday, J.M., Bates, A.E., and Dulvy, N.K. 2012. Thermal tolerance and the global redistribution of animals. Nat. Clim. Chang. *2*, 686–690.

Tanaka, K., Nishizawa, H., Mitamura, H. & Kittawattawanawong, K. 2016. Effects of environmental factors on vocalisation pattern of dugongs revealed by generalised linear model. Techno-Ocean. 55-57.

Thornton, S.J., Toews, S., Burnham, R., Konrad, C.M., Stredulinsky, E., Gavrilchuk, K., Thupaki, P. and Vagle, S. 2022. Areas of elevated risk for vessel-related physical and acoustic impacts on Southern Resident Killer Whale (*Orcinus orca*) critical habitat. DFO Can Sci Advis Sec Res Doc 2022/058. vi + 47p.

Trivers, R.L. 1972. Parental investment and sexual selection. In: Camp- bell B (ed) Sexual selection and descent of man. Aldine, Chicago, pp 136–179

Tucker, C., Sommerville, C. and Wootten, R. 2000. The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Krøyer, 1837) on Atlantic salmon, *Salmo salar* L. J Fish Disease, 23(5): 309-320.

Tyack, P.L. 1986. Population biology, social behavior and communication in whales and dolphins. – Trends Ecol Evol. 1:144–150.

Tyack, P.L. 1997. Development and social functions of signature whistles in bottlenose dolphins *Tursiops truncatus*. Int J Anim Sound Rec 8: 21-46.

Tyack, P.L. & Janik, V.M. 2013. Effects of noise on acoustic signal production in marine mammals. In Animal communication and noise pp 251-271. Eds Brumm, H.

Umeed, R., Attademo, F.L.N., & Bezerra, B. 2018. The influence of age and sex on the vocal repertoire of the Antillean manatee (*Trichechus manatus manatus*) and their responses to call playback. – Mar Mam Sci. 34(3):577-594.

Umeed, R., Lucchini, K. Santos, P.J.P., Attademo, F., Luna, F., Normande, I. and Bezerra, B. 2023. Vocal complexity of Antillean manatees (*Trichechus manatus manatus*). Behaviour.160: 217-256.

Umeed, R., Lucchini, K., Coutinho, P., Santos, P.J.P., Borges, J.C.G., Normande, I., Attademo, F.L.N., Luna, F. & Bezerra, B. 2023. Acoustic interactions between free-living mother-calf Antillean manatees, *Trichechus manatus manatus*. Journal of Ethology https://doi.org/10.1007/s10164-023-00788-z

Van Parijs, S.M., Lydersen, C., Kovacs, K.M. 2002. Sounds produced by individual white whales, *Delphinapterus leucas*, from Svalbard during capture (L). J Acoust Soc Am 113(1): 57-60.

Vasconcelos, R.O., Carriço, R., Ramos, A., Modesto, T., Fonesca, P.J., Amorim, M.P.C. 2012. Vocal behaviour predicts reproductive success in a teleost fish. Behav. Ecol. 23(2): 375-383.

Vincente, J.R., Fonesca, P.J. & Amorim, M.C. 2015. Effects of temperature on sound production in the painted goby *Pomatoschistus pictus*. J Exp Mar Biol Ecol. 473: 1-6.

Von Fersen, L. & Walb, R. (2018). EAZA Best Practice Guidelines for the Antillean manatee. EAZA Executive Office, Amsterdam.

von Schuckmann, K., Le Traon, P.Y., Smith, N., Pascual, A., Brasseur, P., Fennel, K. and Zuo, H. 2018. Copernicus marine service ocean state report. J Oper Ocean, 11:S1-S142.

Wang, Z-T., Nachtigall, P.E., Akamatsu, T., Wang, K-W., Wu, Y-P, Liu, J-C et al. 2015. Passive acoustic monitoring the diel, lunar, seasonal and tidal patterns in the biosonar activity of the Indo-Pacific humpback dolphins (*Sousa chinensis*) in the Pearl River Estuary, China. PLoS ONE, 10(11):e0141807

Wartzock, D., Popper, A, N., Gordon, J. and Merril, J. 2022. Factors affecting the response of marine mammals to acoustic disturbance. Mar Tech Soc J, 37(4):6-15.

Wassmann P, Duarte CM, Agusti S, Sejr MK. 2011. Footprints of climate change in the Arctic marine ecosystem. Global Change Biology 17:1235-1249.

Weilgart, L.S., & Whitehead, H. 1988. Distinctive vocalisations from mature male sperm whales (*Physeter microcephalus*). – Can J Zool. 66(9):1931-1937.

Weiß, B.M., Ladich, F., Spong, F., Symonds, H. 2005. Vocal behaviour of resident killer whale matrilines with newborn calves: The role of family signatures. J Acoust Soc Am, 119(1): 627-635.

Wiggins, S.M., Oleson, E.M., McDonald, M.A. & Hildebrand, J.A. 2005. Blue whale (*Balaenoptera musculus*) diel call patterns offshore of Southern California. Aquat Mamm, 31(2):161-168.

Williams, R., Clark, C.W., Ponirakis, D. and Ashe, E. 2014. Acoustic quality of critical habitats for three threatened whale populations. Anim Cons, 17: 174-185.

Wistos, A. L. 2022. Evaluating environmental factors influencing the movement of four soft-release Antillean manatees (*Trichechus manatus manatus*) in Shipstern Lagoon, Belize. University of Miami.

Wursig B., Dorsey, E.M., Fraker, M.A., Payne, R.S., Richardson, W.J. 1985. Behavior of bowhead whales, *Balaena mysticetus,* summering in the Beaufort Sea: a description. Fish Bull (U.S.) 83: 357-377.

Yuan, J., Wang, Z., Duan, P., Xiao, Y., Zhang, H., Huang, Z., Zhou, R., Wen, H., Wang, K. & Wang, D. 2021. Whistle signature variations among three Indo-Pacific humpback dolphin populations in the South China Sea: a combined effect of the Quiongzhou Strait's geographical barrier function and local ambient noise? Integrative Zoology 16(4): 499-511.

Zeifman, D.M. 2001. An ethological analysis of human infant crying: Answering Tinbergen's four questions. – Develop Psychobiol. 39:265–285.

Zwamborn, E.M.J., Whitehead, H. 2017. Repeated call sequences and behavioural context in long-finned pilot whales off Cape Breton, Nova Scotia, Canada. Bioacoustics 26(2): 169-18

9 Appendices

Appendix A – Article 1: Vocal complexity in Antillean manatees (*Trichechus manatus manatus*).

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Vocal complexity in Antillean manatees (*Trichechus manatus manatus*)

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Abstract

Vocal complexity can be expressed through variations in repertoire size, structure, and individual manatee repertoires. Here we aimed to assess the complexity of the vocal behaviour of Antillean manatees living in captivity (i.e., artificial pools) and in reintroduction enclosures (i.e., natural enclosures placed in an estuarine area). Specifically, we evaluated: (i) the structure of vocalisations to assess whether they had variants; (ii) the variation in call production (rate and pattern) between groups with different configurations; (iii) whether individuality occurred in vocalisation structure. We found four categories of vocalisations, of which two had different variants. Not all study groups produced all call categories and variants. Older and younger males in the reintroduction enclosures had the highest call rates compared to captive females and captive males. The vocal and behavioural patterns differed between groups. Squeak call structure differed between individuals. Such vocal complexity may aid manatees in adapting to their dynamic social and structural environment, facilitating communication.

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Appendix B – Article 2: Acoustic interactions between free-living mother-calf Antillean manatees, *Trichechus manatus manatus*.

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SHORT COMMUNICATION

Acoustic interactions between free-living mother–calf Antillean manatees, *Trichechus manatus manatus*

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Abstract
Acoustic signalling plays a crucial role in mother–calf communication. Here, we describe the acoustic signalling of three free-living Antillean manatee mother and calf pairs and one trio. The mother–calf pairs/trio produced six call types. The call rate varied between the pairs. The pair that experienced human interaction during the recording period had the highest call rate. Additionally, the Squeak vocalisations produced by each mother–calf pair differed structurally. Our results indicate that age and human interaction may affect call rate and suggest the potential production of signature vocalisations by manatee mothers and calves. Our findings add to the current knowledge of manatee acoustic communication.

Keywords Vocal communication · Parental care · Signature vocalisations · Free-living manatees · Acoustic signals

Introduction

Vocal communication between mothers and calves occurs in several marine mammal species (Electronic Supplementary Material 1, 2), including reports for manatees (Hartman 1979; Reynolds III 1981; Mann et al. 2006; O'Shea and Poché 2006; Gerstein et al. 2008). Many studies have attempted to determine the information contained in the acoustic signals exchanged between mothers and calves, highlighting the importance of vocal communication in infant survival. For instance, Florida manatee calves (*Trichechus manatus latirsotris*), vocalise more than mothers (Hartman 1979). Calls from Florida manatee calves have a hill-shaped structure and the mothers' calls have a flatter structure (O'Shea and Poché 2006). Furthermore, calls are considered important in mother–calf individual recognition, with calls preceding movement until mothers and calves are reunited (O'Shea and Poché 2006). Several marine mammal species can produce signature vocalisations (e.g. Electronic Supplementary Material 2), which consist of calls with unique spectral and temporal features that can encode individual or group identity (Shapiro 2006). Signature vocalisations may facilitate mother–offspring contact, individual recognition and reunion (King et al. 2016).

Antillean manatees, *Trichechus manatus manatus*, occur in North-eastern Brazil and are currently under threat of

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